



Sveriges lantbruksuniversitet  
**Fakulteten för veterinärmedicin och husdjursvetenskap**

Swedish University of Agricultural Sciences  
**Faculty of Veterinary Medicine and Animal Science**

# **Use of oxytocin to improve diagnosis of subclinical mastitis caused by *Staphylococcus aureus***



**Lina Jonsson**

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# Use of oxytocin to improve diagnosis of subclinical mastitis caused by *Staphylococcus aureus*

**Lina Jonsson**

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## Abstract

Mastitis, inflammation in the udder tissue, caused by *S. aureus* is a big problem in dairy cattle production. It causes suffering for the cow and curing or replacing the infected cow is costly for the farmer. It is known that beef cattle also suffer from mastitis caused by *S. aureus*. The aseptical methods commonly used to diagnose mastitis in most cases require several milk samples from the same udder quarter. A method where only one sample is necessary would be useful, especially in beef cattle production where handling and milking the animal is difficult. Research has shown that *S. aureus* can change shape into microabscesses, entering the inside of the secretory cell. In this shape it is hidden from the immune system. A high dose of oxytocin could break the secretory cell wall, were the microabscesses are hidden, to shed them in to the milk. The objective of this study was to evaluate if the milk ejecting hormone oxytocin could be used to detect subclinical mastitis caused by *Staphylococcus aureus* (*S. aureus*).

In this trial 14 cows of the breed Swedish red and white were used. Ten produced milk with a somatic cell count (SCC) higher than 250 000 cells per ml milk (HSCC). A control group of four cows producing milk with a SCC lower than 50 000 cells per ml milk (LSCC) were also included. During the trial milkings samples were taken aseptically for bacteriological examination of premilk and postmilk. Premilk samples were taken for SCC analysis. Also composite milk samples for analysis of SCC and composition of fat, protein and lactose were taken during the milking. During the trial the cows were each given a single intramuscular injection with 100 international units of oxytocin.

It was found that the oxytocin injection (OI) resulted in a higher ( $p < 0.0001$ ) amount of milk fat in the milking that followed direct after the injection, compared with all of the other milkings. The OI is known for increasing the amount of residual milk, which has a higher percent of fat. The third milking after the OI, the percent of fat was decreased ( $p < 0.01$ ) compared with all of the other milkings. The amount of protein was higher ( $p < 0.001$ ) at second milking after the OI. At this milking all the cows had a higher ( $p < 0.0001$ ) SCC value and a lower ( $p < 0.0001$ ) percent of lactose. The OI opens up the gap in the tight junctions, which separates the blood from the milk, more somatic cells from the blood can therefore enter the milk and the lactose can move in to the blood.

The HSCC cows had a higher amount of somatic cells than the LSCC group throughout the whole trial period. The two groups both had a SCC peak at the second milking after the OI. Looking at the HSCC cow's udders it was revealed that almost all of the extreme SCC values after the OI came from an udder quarter that had a bacterial infection. There is no result from the bacterial samples that gives support to the theory that a high dose of oxytocin would shed out *S. aureus* that would not be shedded out otherwise. In order to really evaluate the theory behind this trial it would be necessary to look at larger number of cows and also collect aseptic milk samples before the trial starts to be able to select cows with a high SCC that does not have any detected udder pathogen.

## Sammanfattning

Syftet med det här arbetet var att utvärdera om mjölknedsläppningshormonet oxytocin skulle kunna användas som ett diagnostiskt verktyg för att upptäcka subkliniska mastiter som har orsakats av juverpatogenen *Staphylococcus aureus* (*S. aureus*). Juver inflammation, mastit, är ett stort problem för mjölkproduktionen. Korna som drabbas av mastiten lider, då det är smärtsamt för djuret, men det är också kostsamt för lantbrukaren då denne får en lägre produktion mjölk från den drabbade kon samt att det blir en utgift att behandla eller ersätta den drabbade kon. Det har framkommit att även köptraskor drabbas av mastiter orsakade av *S. aureus*. I nuläget används en antiseptisk metod för att diagnostisera mastiten. Denna metod kräver oftast att flera mjölkprover tas, då ett negativt prov kan vara falskt negativt. Då det oftast är väldigt svårt och riskfyllt att ta juverprover från köptraskor behövs en provtagningsmetod där bara ett prov behövs för att säkert kunna ställa en diagnos. Forskning har visat att *S. aureus* kan ändra skepnad och kapsla in sig i s.k. microabscesses. Dessa små kapslar kan inte detekteras av immunförsvaret och är ofta inträngda innanför de sekretoriska cellernas cellväggar. En stor dos av oxytocin skulle kunna öppna upp de sekretoriska cellernas cellväggar och microabscesserna skulle då följa med mjölken ut vid en mjölkning.

Till det här försöket användes 14 kor av rasen svenskt röd och vit boskap (SRB). Tio av dem hade ett celltal som var högre än 250 000 celler per ml mjölk (HSCC). Fyra av dem hade ett celltal som var lägre än 50 000 celler per ml (LSCC), de användes som en kontrollgrupp. Under försöket togs det antiseptiska mjölkprover för bakterieanalys på för- och residualmjölken vid mjölkningarna. Det togs även förmjölkprover för att mäta celltalet och ett samlat mjölkprov från hela mjölkningen för att mäta celltalen och mjölkens sammansättning av fett, protein och laktos. Under försöket blev korna injicerade vid ett tillfälle med 100 internationella enheter av oxytocin intramuskulärt.

Resultatet visar att oxytocininjektionen (OI) ledde till en högre ( $p < 0.0001$ ) andel fett i mjölken från mjölkningen som följde direkt efter OI, jämfört med alla andra mjölkningar. Det är sedan tidigare känt att OI leder till en större mängd residualmjölk än vad som annars är normalt, residualmjölken i sin tur har alltid en högre andel fett än annan mjölk. Den tredje mjölkningen efter OI hade en lägre ( $p < 0.01$ ) andel fett än mjölken vid de övriga mjölkningarna. Mängden protein var högre ( $p < 0.001$ ) vid den andra mjölkningen efter OI. Vid detta mjölkningstillfälle var även celltalet högre ( $p < 0.0001$ ) och laktosen lägre ( $p < 0.0001$ ), jämfört med de andra mjölkningstillfällena. En anledning till detta är att OI öppnar upp de s.k. tight junctions, semipermeabla membran som skiljer blod och mjölk i de sekretoriska cellerna. Fler somatiska celler än normalt kan då ta sig från blodet till mjölken och laktosen går från mjölken till blodet.

Korna i HSCC-gruppen hade ett högre celltal genom hela försöket jämfört med korna i LSCC-gruppen. Båda grupperna hade celltalstoppar vid den andra mjölkningen efter OI. Vissa av korna i HSCC-gruppen hade juverdelar med extremt högt celltal efter OI, denna höjning går i de flesta av fallen att koppla till att den juverdelen hade någon form av juverpatogen. Det finns inget resultat i denna studie som ger stöd åt teorin att en hög dos av oxytocin kan göra så att *S. aureus* som har suttit inkapslat kommer att följa med mjölken ut vid mjölkning. För att verkligen kunna utvärdera den teori som ligger till grund för detta försök är det nödvändigt att använda en större grupp kor än vad som gjordes till detta försök, samt även att ta flera antiseptiska mjölkprover innan försöket startar så bara de kor som har ett högt celltal men ingen annan juverpatogen i juvret används.

## Introduction

Mastitis, inflammation in the udder, is the most common disease in the dairy cattle production. The udder pathogen *Staphylococcus aureus* (*S. aureus*) is one of the most common bacteria that infect the udder and thereby causes inflammation. The bacteria causes permanent damages to the milk secretory cells in the udder tissue, which leads to changed milk composition and reduced milk yield. Once an udder quarter is infected by the bacteria it is very difficult to cure the quarter and get rid of the pathogen, whereby it is very important to detect infected quarters to prevent spreading.

The diagnose of *S. aureus* mastitis in dairy cows is done by aseptically taken milk samples. This method often needs several samples and the accuracy of the method is discussed. One of the reasons that the bacteria is difficult to detect and to get rid of is because it has an ability to hide from the immune system by changing its outer surface or survive in the appearance of microabscesses. A complete emptying of the udder, with a high dose of the milk let down hormone oxytocin, would in the theory brake the secretory cells outer wall and shed its content of microabscessed *S. aureus* in the milk. If this happens in practice one aseptic sample would be enough to diagnose an udder infected with *S. aureus*.

It has been found that a high amount of beef cows also suffers from mastitis caused by *S. aureus*. The infection causes pain to the cow but in beef cattle production it also influence the growth rate on the calf. Beef cows are not handled in the same extension as dairy cows and they are not used to be milked by hand or machine. To take aseptically milk samples from beef cows is very unusual due to the reasons mentioned and the extension and knowledge of beef cows' mastitis is very unexplored. A more accurate and easy sampling is therefore necessary to be able to detect and diagnose the *S. aureus* caused mastitis in beef cows.

## Aim

The aim with this study was to find out if cows with a suspected *S. aureus* infection would release microabscesses *S. aureus* in the milk when a high dose of oxytocin was given. The oxytocin dose should be big enough to create physical changes in the secretory epithelium of the udder tissue.

The question to be answered is if one high dose of oxytocin can be used as a diagnostic tool of subclinical mastitis so that one aseptically milk sample can be enough to make a diagnose.



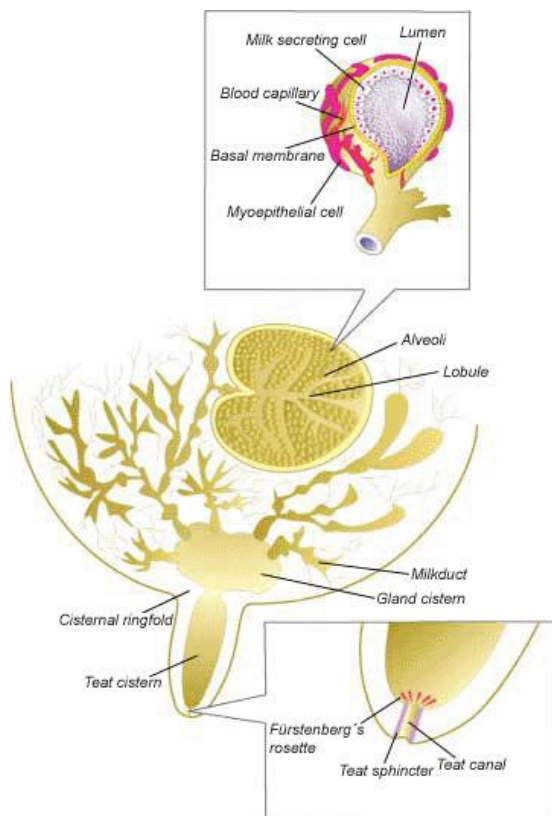
## Literature review

The *S. aureus* bacteria infects the udder and causes changes of the structure. It is therefore important to understand the anatomy of the udder. The structure of the udder also plays an important function in how the bacteria get in to the milk secreting glands.

## Udder physiology

### *The structure of the udder*

The cow udder is divided into separate udder quarters, one for each teat. The teat tip consists of a teat sphincter and the Fürstenberg's rosette, with smooth muscle that can close the teat canal. This part of the udder function as an important protection shield that prevent foreign particles from enter the inside of the udder. The base of the teat has a cisternal ringfold. The inside of the teat, between the tip and the base is the teat cistern, see fig.1. The gland's cistern is located above the teat cistern. The gland cistern is connected to the milk secreting cells by a duct system. This system starts with larger ducts that divide up in a net of smaller ducts. These ducts lead to the alveolus and the milk secreting cells. The alveolus is surrounded by a thin layer of connective tissue that keeps the alveolus together. The connective tissue also contains blood vessels. The connective tissue forms the alveolus in to larger groups, lobule. The milk ducts from the alveolus in the lobule are connected to larger collective duct. The lobules are also formed into larger groups by connective tissue, these are called lobe. The milk duct from each lobule is gathered into larger milk ducts with a thicker epithelium layer and they end up in the gland cistern (Tanhuanpää 1995).



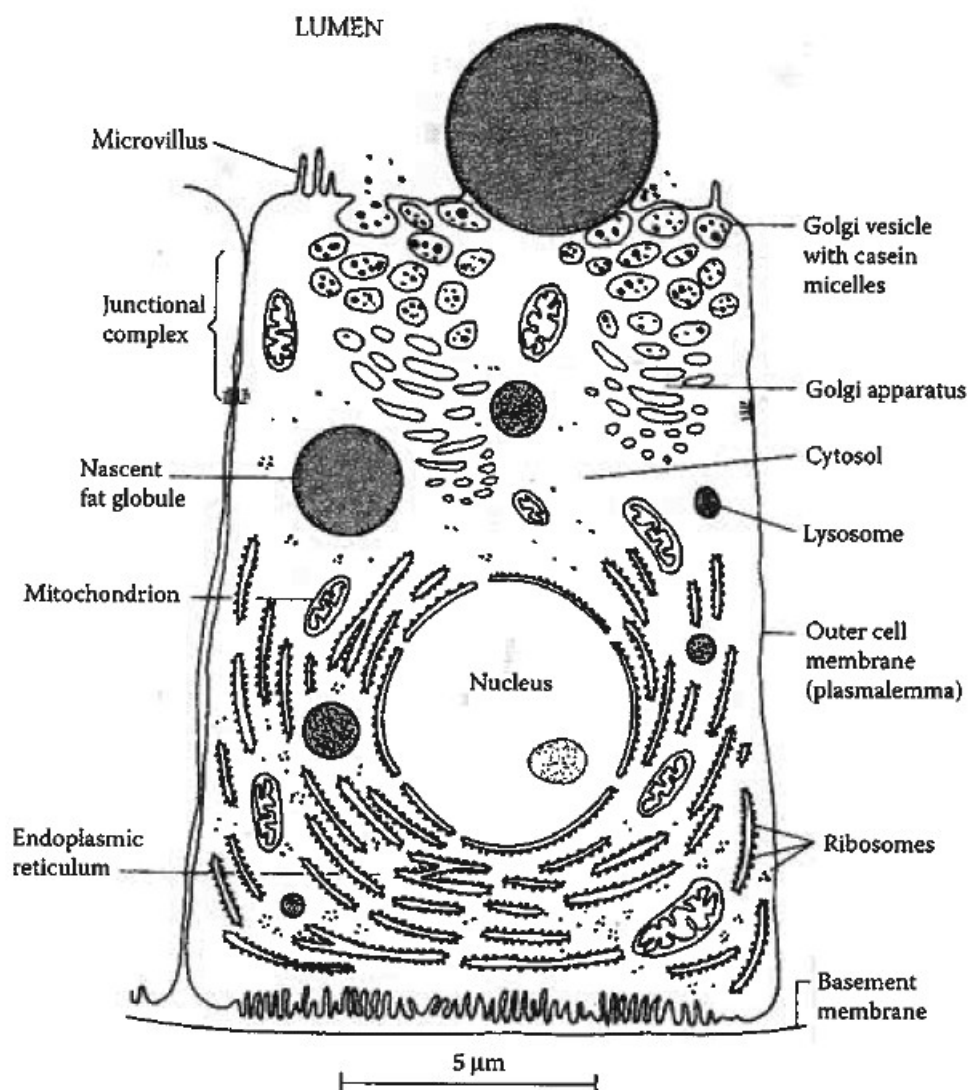
**Fig. 1.** Picture of the mammary gland with the teat cistern and alveolus cell magnified (DeLaval, 2009).

### *Defense mechanisms*

The cow has several ways of protecting the udder from microorganisms, the skin and teat canal function as the first defense mechanism. The Fürstenberg's rosette in the teat tip closes about two hours after milking. The renewed off skin cells at the teat, and the milk flow, are during milking also functioning as defense mechanisms. This prevents the bacteria from physical enter the mammary gland. The keratinized skin layer on the outside of the teat contains antibacterial fatty acids and base antibacterial proteins. The second line of defense includes the neutrophils and macrophages. The neutrophils and macrophages recognize the bacteria and destroys them by phagocytose. The neutrophils are considered to be the most important defense mechanism in the udder. The bigger macrophages alarm the rest of the immune system, the adaptive cells. (Sandholm & Korhonen 1995). The adaptive immunity with lymphocytes as t- and b-cells, belongs to the third line of defense (Grönlund 2004).

### *Milk secretion and composition*

The spherical alveolus consists of a single layer of secretory alveolar cells, se fig. 2 (Tanhuanpää 1995). At the basal membrane of the cell is the blood capillary. At the apical side is the alveolus lumen with milk. The alveolar cell is provided with nutrients from the blood plasma. In the cell different milk components are synthesized and milk is formed. (Kaartinen 1995). The main components in milk are water, fat, lactose and protein. Milk from a healthy udder has the average composition as shown in table 1 (Walstra *et al.* 1999).



**Fig. 2.** Picture of the mammary epithelial cell (Walstra *et al.* 1999).

**Table 1.** The main components in milk

Component	Average content in milk (%)
Water	87,1
Solids-not-fat	8,9
Lactose	4,6
Fat	4,0
Protein	3,3
Casein	2,6
Mineral substances	0,7
Organic acids	0,17
Miscellaneous	0,15

(Modified after Walstra *et al.* 1999).

Lactose is synthesized from glucose and galactose, assisted by the protein  $\alpha$ -lactalbumin (Walstra *et al.* 1999). This synthesis takes place in the Golgi apparatus. The lactose is transported in vacuoles to the apical membrane of the alveolar cell. When the vacuole reaches the membrane the content is released in the milk by exocytosis. Lactose works as an osmotic gradient. The basal membrane in the alveolar cell is water permeable. This makes it possible for the water to enter the alveolar cell from the blood plasma. The vacuole filled with lactose is expanding with intruding water until the osmotic pressure is equal between the vacuole and the secreting cell (Kaartinen 1995). The amount of milk is by this regulated by the amount of synthesized lactose (Larson 1985). An ion flow follows with the water from the blood plasma (Kaartinen 1995). A  $\text{Na}^+$ ,  $\text{K}^+$ -pump transports sodium back to the blood and potassium into the alveolar, leading to a higher concentration of potassium in milk than in the blood (Larson 1985).

Milk contains different types of proteins, see table 2. There are two major classes, casein and whey proteins. The whey proteins is also named serum proteins. Most of the protein is synthesized in the rough endoplasmatic reticulum in the alveolar cell. After the synthesis the protein molecule enters the Golgi apparatus, where it is packed into vacuoles that move to the apical membrane of the cell (Kaartinen 1995).  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, serum albumin and immunoglobulins are serum proteins. Serum albumin enters the alveolar cell from the blood and immunoglobulins are synthesized by antibodies (Walstra *et al.* 1999). The amount of protein synthesized in the alveolar cell is difficult to influence by the feeding of the cow, since the protein and lactose synthesis are closely connected in the cell synthesis (Kaartinen 1995).

**Table 2.** Milk proteins

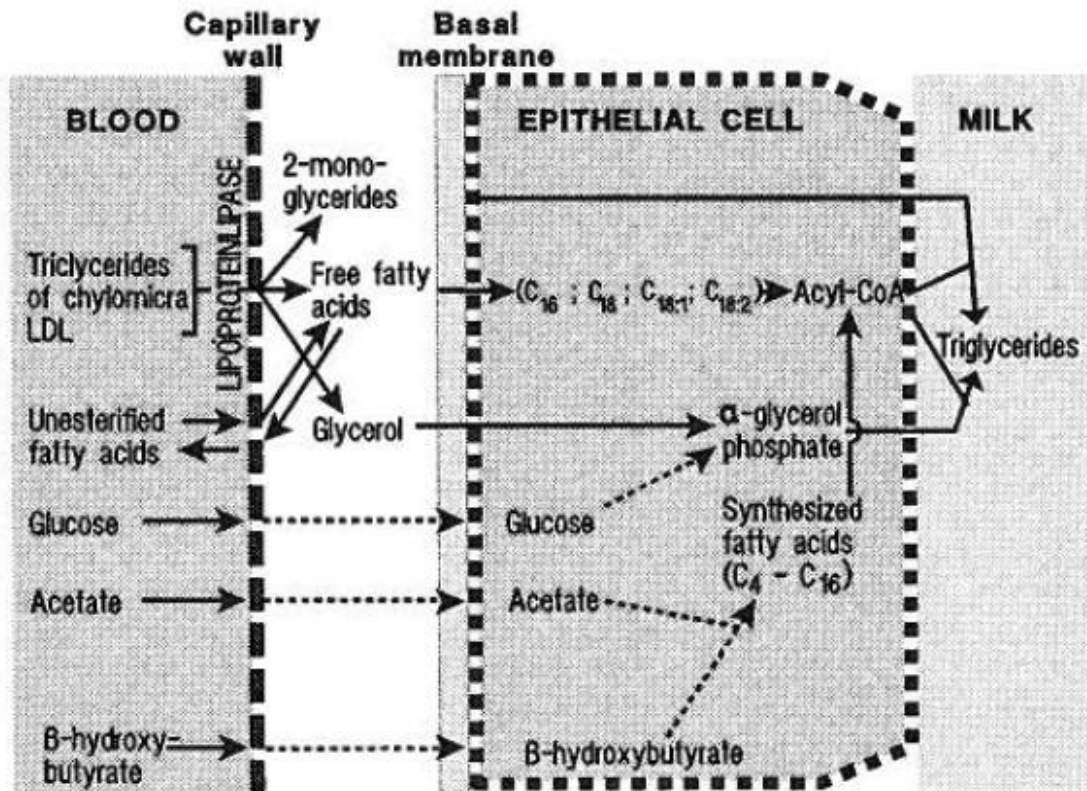
Name of protein	Concentration (% w/w)
Caseins	2,6
$\beta$ -lactoglobulin	0,32
$\alpha$ -lactalbumin	0,12
Lactoferrin	0,01
Serum albumin	0,04
Immunoglobulins	0,08

(Modified after Walstra *et al.* 1999).

The fat in milk consists of droplets surrounded by a membrane. The droplet consists of triglycerides and the membrane consists of phospholipids and cholesterol. The triglycerides are synthesized from two different sources (Fig. 3) (Kaartinen 1995).

One source is from chylomicrons and low density lipoprotein in the blood plasma. When these enter the blood capillary wall the enzyme esterase breaks them down to mono-, diglycerides, glycerol and free fatty acids, so they can enter the alveolar cell. In the cell the fatty acids substrate are synthesized into fatty acid chains, containing 16 or 18 carbons (Kaartinen 1995). Triglycerides can also be synthesized from *de novo synthesis*. It takes place in the epithelial cell in the mammary gland. Smaller compounds e.g. glucose,  $\beta$ -hydroxybutyrate and acetate is synthesized into triglycerides. The fatty acids from this process contain 16 carbons or less. Since the fatty acids have different length depending on which metabolic compound it derives from, the milk fat composition reflect the cow's diet (Sjaastad, 2003; Kaartinen 1995).

The endoplasmic reticulum in the alveolar cell is synthesizing the milk fat and makes it into fat droplets of different size. These droplets are moving to the apical part of the alveolar cell. The drops melts together during this transport, hence droplets close to the apical part is much bigger than the newly synthesized. When the droplets reach the apical membrane they are secreted by endocytosis. The fat droplet becomes enclosed by the apical membrane when it enters the alveoli lumen (Kaartinen 1995).



**Fig. 3.** Milk fat synthesis (Kaartinen 1995).

### *Tight junctions*

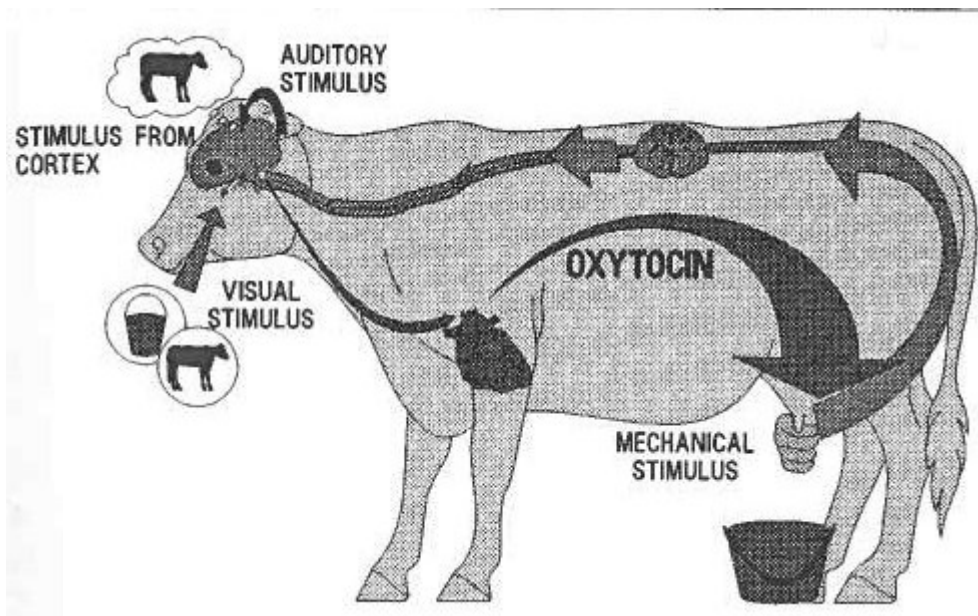
The alveolar cells are kept together by junctional complex, which consist of adherent junction and desmosomes (Nguyen & Neville, 1998). This complex creates tight junctions that under normal circumstances creates an impermeable barrier and prevent particles from the blood plasma to enter the alveolus lumen, lactose and milk proteins to enter the blood stream (Larson 1985, Nguyen *et al.* 1998). The tight junction has an important function in the secretion of milk and a leaky tight junction is negative for the milk production (Nguyen & Neville, 1998). The tight junction becomes natural leaky in the end of the lactation and the milk composition changes throughout the lactation (Auldist *et al.* 1996, Nguyen & Neville, 1998). The leaky tight junction during late pregnancy will after the parturition form a tight barrier. The hormone cortisol has an important role in making the tight junction less permeable (Nguyen & Neville, 1998). This is showed by changes in milk composition. A higher cortisol level gives increased sodium and reduced potassium levels in the milk (Allen 1990). The tight junction can also become leaky by the entrance of bacteria in the udder or by influence of the hormone oxytocin. These important changes of the tight junctions will be explained more in the following chapter.

## Oxytocin and milk let down reflex

### *Myoepithelial cell and oxytocin*

The main part of the milk is stored in the alveolus. Only around 20 % of the milk is stored in the cistern. Therefore it is important to empty the alveolus in order to get all milk from the udder (Bruckmaier & Blum 1998). The alveoli are surrounded by myoepithelial cells. They consist of smooth muscle cells arranged like a net. During contraction they diminish the alveolus lumen, see fig. 1 (Sandholm a 1995). The hormone oxytocin causes contraction of the myoepithelial cells (Bruckmaier & Blum 1998). Myoepithelial cells are also present around the milk ducts. During a contraction of these myoepithelial cells the milk duct length is shortened. The milk is by these contractions pressed from the alveoli into the milk duct and further down to the gland cistern (Sandholm a 1995).

The hormone oxytocin is released from the posterior pituitary after a milking stimulus (Fig. 4). From the posterior pituitary oxytocin is released after visual, auditory or mechanical stimuli, the so called milk ejection reflex. Milk ejection reflex is evoked by the suckling calf, the noise from a milking machine or a milker cleaning the teats (Sandholm a 1995). Oxytocin is released in the blood after 1-2 min after the start of stimulation (Bruckmaier *et al.* 1994). For dairy cows the stimulation can consist of precleaning, sight and sound of the milking equipment. The amount of oxytocin that is being released in the blood varies with the individual cow and also with the situation. A stressful situation that generates adrenalin depresses the milk ejection reflex (Sandholm a 1995). Oxytocin is also functioning as a hormone in other physiological functions (Schams 1992).



**Fig. 4.** Different stimuli for releasing oxytocin (Sandholm a 1995).

## Mastitis and Somatic Cell Count

### *Mastitis*

An inflammation in the mammary gland is called mastitis (Harmon 1999). This inflammation in the mammary tissue could be due to several reasons. One of them is an infection of bacterial colonization. It could also be done by an incorrect machine milking that harms the udder, a teat injury or a squishing or a kick on the udder. A damaged mammary tissue is less resistant against a bacterial attack than a solid (Sandholm b. 1995).

### *Somatic cell count*

By measuring the somatic cell count (SCC) in milk it is possible to estimate the udder health (Schepers *et al.* 1997). The somatic cell consists mostly of cells from the immune system as macrophages, lymphocytes and neutrophils, but also cell such as epithelial cells (Pillai *et al.* 2001). When the SCC is high it constitutes to a large part of neutrophils (Sandholm & Korhonen 1995). The somatic cells reflect the inflammatory response in the udder from a trigger to the immune system, as an intramammary infection. The SCC increases with the infection rate. The SCC can therefore be used to detect udder quarters with inflammation which can be caused by an infection. A SCC of 250 000 cells/ml is often used as a threshold value between a healthy and an infected udder. Although researchers suggest that a SCC around 50 000 cells/ml is considered as an udder quarter without any infection (Schukken *et al.* 2003). The SCC increases naturally in the end of the lactation (Sandholm b 1995; Auldist *et al.* 1996).

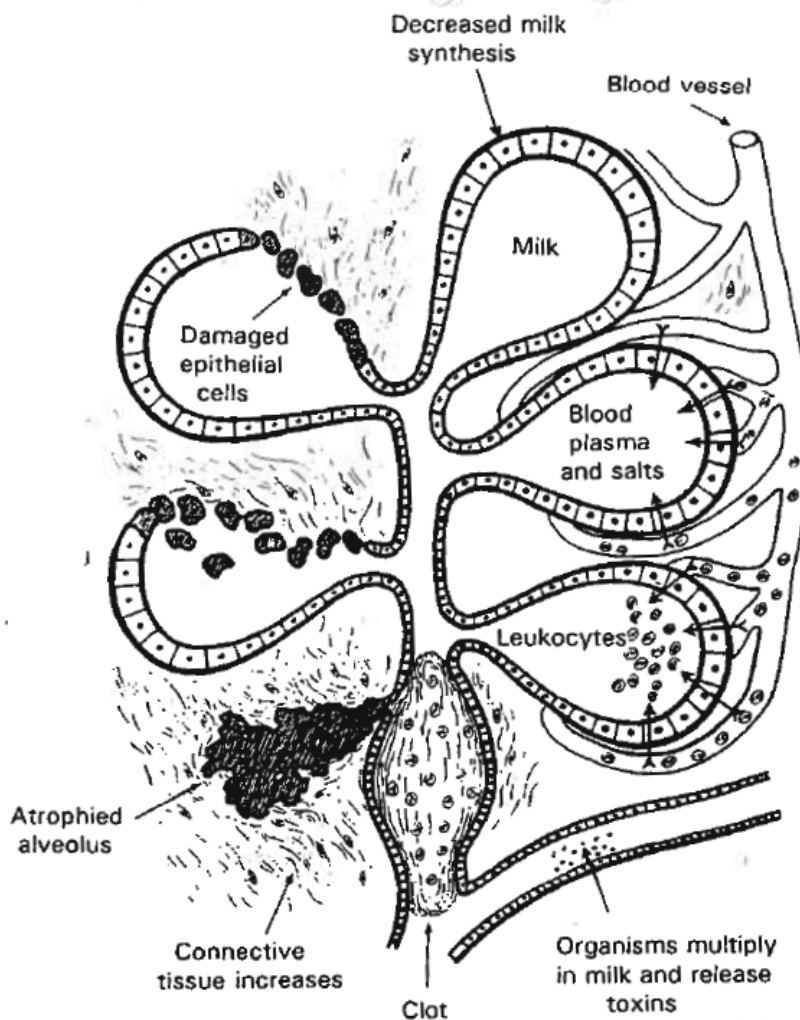
### *Changes in the tight junctions during an infection*

In the beginning of an intramammary infection,, the somatic cells in the mammary glands signals to the white blood cells in the blood stream that something unknown has entered the gland (Burvenich 1994). This recognition is mostly done by macrophages. The circulation in the area and the permeability of the capillaries increase, due to a contraction of the endothelial cells (Sandholm b 1995). The tight junctions become leaky (Stelwagen *et al.* 1999). Through these gaps plasma and proteins from the blood can enter in to the alveolar lumen. This results in a large flow of mostly neutrophils from the blood to the milk (Burvenich 1994). These phagocytic cells attacks and kill the bacteria. When the infection is over the SCC returns to normal level, this takes a couple of weeks (Van Werven *et al.* 1997). This is called clinical mastitis and causes an increased number of somatic cells, changes in the milk composition, swelling and pain in the udder and reduction in milk production. If the immune defense mechanisms fail to eliminate the bacteria the infection becomes chronic. The SCC will stay on a high level, the infection goes in to a subclinical stage but with clinical periods. During a subclinical mastitis the udder looks normal but the milk production is decreased and the milk composition is changed. Since the bacteria still exists in the udder, they are secreted out in the milk (Harmon 1994). The macrophage's response to an infection is the same as with any type of inflammation.

The permeability in the barrier between the blood and alveolar is reduced during an infection, this is indicated by higher sodium and plasma protein levels in the milk. The change in permeability is due to two main reasons. The first is because the immune defense system reacts on the infection and enters the alveolar cell from the blood plasma. In order to do that the tight junctions have to open up to let the defense mechanism in. The second reason is due to the tissue damage that the infection can create in the mammary epithelium which can lead to changes in milk composition (Nguyen & Neville, 1998). An infection by microorganisms or a high intramammary pressure due to interruption during milking can also make the tight

junctions leakier. This is partly because the epithelial cell gets damaged, see fig. 5 (Larson 1985).

At the drying-off the udder is going through a sterile inflammation in order to prepare the udder for cleaning and reparation. Hormones from the upcoming pregnancy are an important factor in this process, in particularly oestrogen. In the beginning of a lactation, the tight junctions are still permeable and substances from the blood can leak between the blood and milk barriers. At the end of lactation and drying-off it might be difficult to distinguish a bacterial infection since the somatic cells are high, the ion concentration different and the lactose levels are lower compared to the mid-lactation (Sandholm b 1995). In order to find a quarter with an inflammation caused by a bacterial infection a comparison between the four quarters might in this case be a better way than to look at the total SCC.



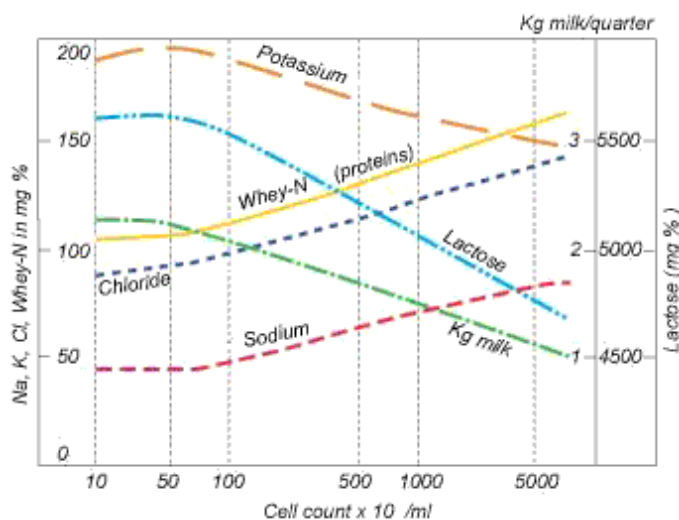
**Fig. 5.** The epithelial cells become damage and get a decreased synthesis from a bacterial inflammation (Harmon 1999).



### *What happens to the milk during an infection?*

Due to the influx of substance from the blood to the alveolar lumen, through the cellular gaps the ion composition is changed during an inflammation (Sandholm b 1995). The sodium and chlorine concentration is increased, the milk becomes more alkaline (Kitchen 1981; Sandholm b 1995; Harmon 1994). More enzymes are released into the lumen and the synthesis of casein, fat and lactose is degraded (Sandholm b 1995). The decrease of the synthesis is because the secreting cells get damage by toxins from the bacteria and by enzymes from damaged cells (Kitchen 1981; Fox *et al.* 1985). As mentioned before it also causes an enhanced SCC, changed ion composition, increased amount of enzymes and a degradation of the synthesis of casein, fat and lactose, see fig. 6 (Sandholm b 1995).

When exogenous oxytocin is given the SCC in a healthy udder is doubled, whereas an udder with chronic mastitis the SCC is increased fourfold (Sandholm a 1995). Oxytocin opens up the tight junctions between the alveolar cells (Allen 1990). The leaky tight junctions make a passage between the milk and the blood possible. This can be measured by an increased concentration of somatic cells and sodium, whereas the amount of lactose and potassium in the milk is decreased when oxytocin is being injected (Linzell *et al.* 1975; Allen 1990; Sandholm a 1995).



**Fig. 6.** When SCC increase the milk yield and milk composition is changed (Korhonen & Kaartinen 1995).

### *Mastitic pathogens*

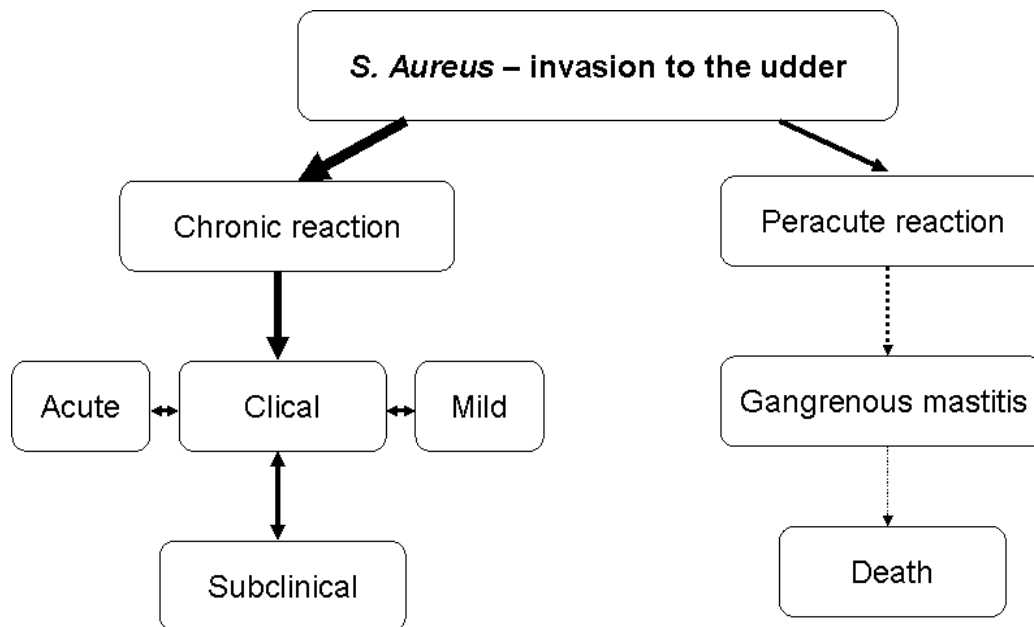
The pathogens that causes mastitis can be divided into two groups; minor or major pathogens. Minor pathogens are coagulase-negative staphylococci and *Corynebacterium*. They usually cause clinical mastitis that does not become chronic. To the group of major pathogens of environmental origin is *Staphylococcus aureus*, *Streptococcus agalactiae*, coliforms, streptococci and enterococci included. An intramammary infection by these pathogens is likely to develop a chronic mastitis. These environmental bacteria are found in the soil, manure and bedding. Hence the bacteria are spread during milking, most of the new infections happen between the milking occasions (Harmon 1994). It is bacteria from the genera *Staphylococcus* and *Streptococcus* that causes the most intramammary infections in the Nordic countries, both clinical and sub-clinical mastitis (Pyörälä 1995).

## Staphylococcus aureus

*S. aureus* is responsible for around 30-40 % of the sub-clinical mastitis (Pyörälä 1995). The SCC increases more by an infection from *S. aureus* compared with an infection from coagulase-negative staphylococci (Schepers *et al.* 1997).

Since the Staphylococcal bacteria exist in the cow's environment it is also a part of the microflora at the skin (Pyörälä 1995). Genotyping *S. aureus* from infected udder quarters showed that one udder quarter can have several strains of *S. aureus* at the same time. It was also revealed that most of the infected udder quarters on the same farm had a limited number of genotypes, which indicates that *S. aureus* is spread from quarter to quarter (Young *et al.* 2001). It is more common that the cow get a Staphylococcal infection close after the calving or at the drying-off. A wound at the teat end can easily get colonised by the bacteria. During milking the bacteria can enter the udder. The *S. aureus* spreads quickly in the udder and the neutrophils tries to reduce them. If the *S. aureus* accumulates fast, the response from the neutrophils is too weak, the bacterial infection can develop into a preacute mastitis. The tissue gets oedema and necrosis, the cow gets a fever and loses her appetite. This can in rare cases be deadly, see fig.7. (Pyörälä 1995). Milk with high SCC, caused by an *S. aureus* infection, has a higher proportion of neutrophils compared to milk from an uninfected udder quarter (Sordillo *et al.* 1989; Piccinini *et al.* 1999).

A normal development by a *S. aureus* bacteria has often more drastic symptoms if it occurs around calving, than around the dry-off. The SCC increase, the milk becomes flaky, and some times blood is found in the milk.

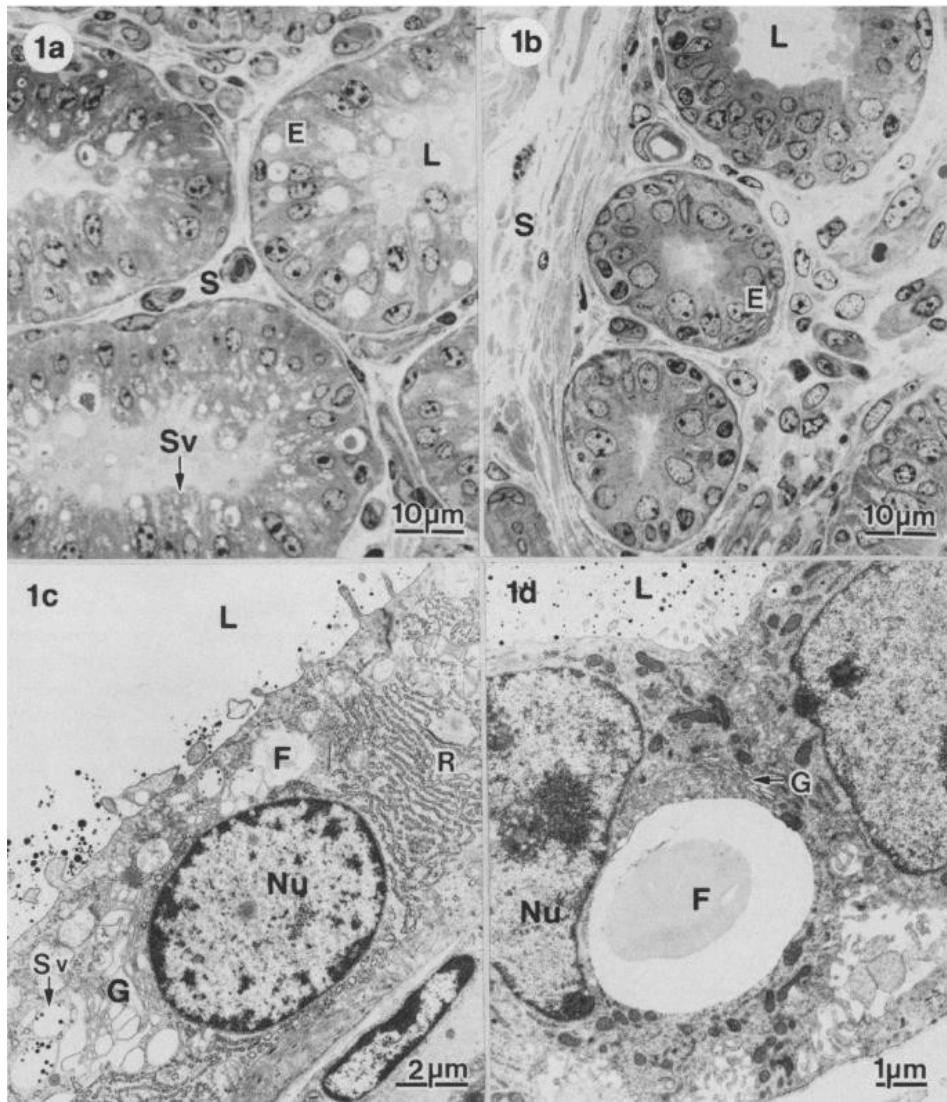


**Fig. 7.** Two different forms of a *S. aureus* infection (modified from Pyörälä 1995).

The bacteria *S. aureus* is a gram-positive Cocci of the family Micrococcaceae of four genera: Micrococcus, Planococcus, Stomatococcus and Staphylococcus. The *Staphylococcus* can ferment glucose anaerobe, have a negative response to oxidase and benzidine tests. The *Staphylococcus aureus* is found on the skin and in the nose of animals and humans. The colors of the colonies are grey with a tone of yellow. It is facultative anaerobe. Almost all strains produces coagulases which can be used for the identification on blood agar plates. The bacteria also have the ability to produce catalase which prevent it from being oxidative damaged by neutrophils. This is done by the catalase ability to cleave the  $H_2O_2$  to  $H_2O$  and  $O_2$  (Burvenich *et al.* 1994). *S. aureus* can produce four different haemolysins (exotoxins)  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ , where the different strains can produce at least one of the four haemolysins (Gilmour & Rowe 1981). These haemolysins are heat-stable and causes lysis of red blood cells *in vitro* (Gilmour & Rowe 1981; Walstra *et al.* 1999). The bacteria can grow at 15-45°C, although they grow the ultimate temperature are 30-37°C (Gilmour & Rowe 1981). The growth of the bacteria in bulk milk can be slowed down by reducing the temperature of the milk. Low pasteurization kills the bacteria but not the exotoxins (Walstra *et al.* 1999).

The *S. aureus* can exist inside epithelial cell, neutrophils and macrophages (Pyörälä 1995). Almedia *et al.* (1996) has showed that when *S. aureus* intrudes the mammary epithelial cell, it is probably done by a contribution by the epithelial cell itself. The *S. aureus* is taken in to the cell by the cells F-actin microfilaments. It was also showed that bacteria in membrane-bound vacuoles inside the epithelial cell were able to divide, and thereby increase in number. This phenomenon makes it more difficult for the immune system to find the bacteria (Pyörälä 1995; Struder *et al.* 2008). When the *S. aureus* has been phagocytised and developed into microabscesses, it can still survive inside and be shedded out in the milk (Craven & Anderson 1984). The bacteria can also escape phagocytosis and by this become chronically (Pyörälä 1995; Struder *et al.* 2008).

In a study of udder quarters development during parturition, a comparison between quarters infected by *S. aureus* was compared with uninfected quarters, see fig. 8. It was found that the development of the infected quarters during the last part of the gestation was decreased. The secretory ability was lower, the luminal space was reduced and the stromal area was larger, compared to the uninfected quarter tissue. The infected quarters had lower active secretory epithelia, higher concentration of  $\alpha$ -lactalbumin in blood and an increased SCC, which indicates leaky gaps or damage and destroyed epithelial cells (Sordillo *et al.* 1989).



**Fig. 8.** A comparison between udder quarters infected with *S. aureus* and uninfected quarters. 1a. An uninfected mammary tissue. The stromal area (S) is only a small part of the total tissue area. The active epithelial cell (E) is fully developed with several secretory vesicles (Sv). 1b. Mammary tissue from a infected quarter. The stromal area take up more space around the cells, causing a reduction of the luminal area (L). Non active epithelial cells (E) closely packed with the wizened alveoli. 1c. A part of an uninfected alveoli cell. The nucleus (N) has a regular rounded shape, a normal sized fat droplet (F), large and well developed rough endoplasmic reticulum (R) and Golgi apparatus (G) with secretory vesicles (Sv). 1d. Infected tissue. The nucleus (Nu) has an irregular form, the fat droplet is enlarged and the Golgi apparatus is compressed (from Sordillo et al. 1989).

It has also been found that *S. aureus* is able to exist in different forms. One form is existing as small colonies of an L-form. These L-forms lack cell walls and are by this resistant against antibiotics that work by destroying the cell wall of the bacteria (Pyörälä 1995; Owens & Nickerson, 1989). The L-form is by this a way for the bacteria to wait out an antibiotic treatment (Owens 1987). A study made *in situ* showed that *S. aureus* in an L-form can by penicillin treatment go in to a form that lack cell wall that still could grow in to new L-form colonies (Owens & Nickerson, 1989). A small-colony variants (SCV) form is also found. When the *S. aureus* switches in to the SCV form it can survive better in a non-phagocytic cell than the normal form are able to (Brouillette et al. 2004).

### *Sampling methods*

When the *S. aureus* exists in the mammary epithelial cells it is shedding out in the milk. The shedding is found to be in a cyclic pattern, following a sinusoidal curve and individual for each udder quarter and cow. It seems to have a half-phase duration of 6,5 days where it reaches a shedding maximum, after which the shedding decreases. This sinusoidal pattern can be due to a synchronized struggle with the immune system (Studer *et al.* 2008). When the SCC is high, the amount of bacteria in the milk is low, and when the bacteria counts are high, the SCC are low (Daley *et al.* 1991). These changes in shedding pattern makes it more difficult to take an accurate sample. The risk of a false negative result is increased if the glands have a low shedding cycle or if the immune system has depressed the amount of bacteria in the udder at the day of sampling (Sears *et al.* 1990; Daley *et al.* 1991). Studies have shown that one sample result in 74,5 % detection of the case to a bacteriological culture of *S. aureus*. If the sampling were prolonged with two or three consecutive samples the sensitivity increased to 94 % and 98 % respectively. Considering the shedding pattern of *S. aureus* a subsequent sampling is better than a repeated sampling during the same milking occasion. It was also found that an experimentally infected udder quarter can give negative samples throughout a whole cycle (Sears *et al.* 1990). When the sensitivity of samples taken from premilk and postmilk are compared, premilk gave a sensitivity of 91 % and postmilk 81%. Although multiple isolates were shown to be more common from premilk samples and the theory that a postmilk sample comes from a flushed/rinsed udder and by this cleaner teat canals, makes the author to recommend postmilked samples (Sears *et al.* 1991). Another factor to have in mind when a bacteriology milk sample is taken is if it should be stored frozen or not. It has been shown that by freezing the sample disturbed macrophages and neutrophils and phagocytized bacteria can be realized and detected (Buelow *et al.* 1996; Godden *et al.* 2002). It has also been shown that freezing of postmilked samples results in more positive *S. aureus* samples than a fresh postmilked sample does (Godden *et al.* 2002). Frozen premilking sample have also been used for the purpose to test their sensitivity of finding *S. aureus* with a high sensitivity as a result (Buelow *et al.* 1996).

### *Treatment of udders infected with S. aureus*

It is difficult to cure a quarter that is infected with *S. aureus*. As mentioned above, the bacteria have several ways of escaping antibiotics. The chances of getting rid of the bacteria increases if it is treated fast and if it is a young animal (Pyörälä 1995; Sears & McCarthy, 2003). But considering the fact that it is difficult to find a sampling method that has a high accuracy makes it even more difficult to evaluate if a treatment really works.

Several ways of treating a *S. aureus* infected quarter exists. The most common ways are by antibiotics given during the lactation, dry cow therapy and oxytocin. The most frequent used method for clinical mastitis is antibiotics. There have been studies made on clinical mastitis that compare antibiotics with oxytocin. They did not show any differences on the effect between the antibiotics or the oxytocin treated quarters (Guterbock *et al.* 1993; Knight *et al.* 2000). The cure rate of antibiotics on a *S. aureus* infected quarter varies a lot. It is depending on the type of antibiotic but also the length of the treatment (Barkema *et al.* 2006; Owens *et al.* 1988).

Dry-cow treatment has a better cure rate than treating the cow with a short antibiotic cure during the lactation. This can be explained by the fact that in a dry-cow treatment the antibiotics is in the udder for a longer time, it is not being milked out, and the udders own defense mechanisms are extra active in the first period of the dry time (Sandholm & Pyörälä, 1995; Sears & McCarthy, 2003).

Another way of achieving a healthy udder is by vaccinating the cow against *S. aureus*. But even though, the understanding about the immune system and the bacteria is increasing all the time, and the technologies for mastitis vaccine is developing, there is not any commonly used vaccine program against mastitis (Sordillo *et al.* 1997; Barkema *et al.* 2006). One theory to why the vaccine have a low cure rate is that is strain specific and *S. aureus* exist in so many different stains (Barkema *et al.* 2006).

A chronic mastitis caused by *S. aureus* costs a lot of money for the farmer. This is due to several reasons; the cow produce less milk, the farmer gets less paid per liters of milk due to the high SCC and the replacement cost for a new cow, to mention some of them (Schepers & Dijkhuizen, 1991). There is also a question if there are any economic benefits of treating the cow during the lactation, or wait until the dry period. The veterinary and medicine costs and that the milk have to be discarded during the treatment and the following days, or that the cow has the chronic mastitis the entire lactation out (Swinkels *et al.* 2005).

## Mastitis in beef cows

The prevalence and effect of subclinical mastitis in beef cows is not so well documented as it is in dairy cows (Simpson *et al.* 1995). The studies that have been made on the topic show a varying percent of infected animals. Lents *et al.* (2008) found that 28.8 % of the examined cows had some of the quarters infected with a mastitis causing bacteria. Other studies show higher number; 32, 37, 37 and 66 % of the cows were infected (Simpson *et al.* 1995; Watts *et al.* 1986; Lents *et al.* 2002; Paape *et al.* 2000). *S. aureus* is one of the most common udder pathogen in beef cows (Watts *et al.* 1986; Newman *et al.* 1991; Simpson *et al.* 1995; Lents *et al.* 2008). Beef cows with some kind of udder pathogen had a higher SCC than the uninfected cows. Cows with *S. aureus* infection in some of the quarters get particular high SCC (Newman *et al.* 1991; Lents *et al.* 2008).

One part of the problem, that so few studies have been made, is that it is not so easy to take accurate milk samples from beef cows. This is because they are not used to being milked by hand or machine and to take a sample from an untamed beef cow is very dangerous. As described earlier one aseptically taken milk sample might not be enough to detect mastitis. A more accurate method is there for needed.

Calves from cows with high SCC grow less than calves from cows with low SCC (Watts *et al.* 1986; Newman *et al.* 1991; Simpson *et al.* 1995). The weight gain for calves is also dependent on which type of bacteria that causes the infection. A calf to a cow with a quarter infected by *S. aureus* grown less than calves to cows that has uninfected quarter infected (Watts *et al.* 1986; Newman *et al.* 1991). Beef cow quarters that are infected has shown to have a lower amount of fat, protein and lactose compared to uninfected quarters. This change in composition is even more obvious if the quarter is infected by *S. aureus* (Paape *et al.* 2000). The fact that a beef cow with an infected udder produces less milk and with a changed composition does not effect the young calf so much, since it does not use the full capacity of the udder. Although when the calf becomes older this capacity limitation becomes more obvious in the weight gain for the calf (Newman *et al.* 1991). The amount of infected quarters on the same cow has also an impact on the weight gain for the calves (Watts *et al.* 1986). The number of infected cows has been found to increase with the parity. The pathogen does not spread with milking equipment as it does for a milking cow, since it is not used on the beef cows, but there is no teat dipping after a calf has stopped suckling, and the calves might also spread the pathogen between the quarters (Paape *et al.* 2000). Research has been done on the effect of dry treatment on infected quarters. Calves to cows that had been treated grow better than calves to cows that had an infection but had not been treated for it. The treatment had not an effect on quarters infected by *S. aureus* (Lents *et al.* 2008).

Seen from an animal welfare point of view it is not only the calf life that is affected. Having a subclinical untreated mastitis that also comes in to acute phases is thought to be very painful for the cow (Kemp *et al.* 2008). It is therefor important to find a method to detect subclinical mastitis at beef cows, without causing unnecessary stress to the animal or danger for humans.

## Material and methods

### Animals and housing

In this study dairy cows of the breed Swedish red and white were used. They were housed in a conventional tied up barn at Kungsängen research center in Uppsala, and at Jälla farm in Uppsala, Sweden. The cows were milked conventionally twice a day, at 7 am and 16 pm. They were fed with silage and concentrate in the amounts according to the Swedish recommendation for feeding (Spörnly, 2003).

In total 14 cows from Kungsängen and Jälla dairy herds participated, twelve cows from Kungsängen and 2 from Jälla. They were divided into two groups;

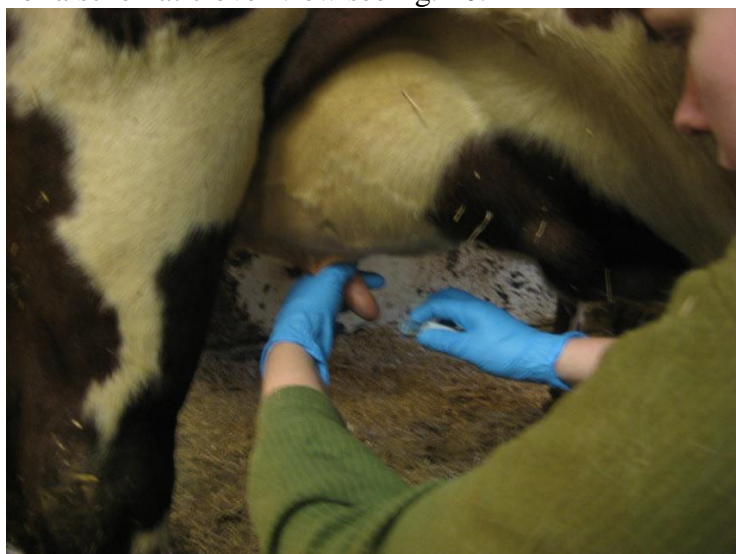
One group consisting of 10 cows that had a SCC higher than 250 000 cells per ml milk or cows that had a SCC lower than 250 000 cells per ml milk, but had a previous history of *S. aureus* at some of their udder quarters. This group is called high somatic cell count (HSCC). The two cows from Jälla belonged to this group.

The other group consisted of 4 cows that had a SCC lower than 50 000 cells per ml milk. These cows were used as a control group and were not allowed to have had a known history of *S. aureus* infection. This group is called low somatic cell count (LSCC).

The cows in the two groups were in lactation stage between 30 days and 300 days in lactation when the treatment started.

### Performance and sample collection

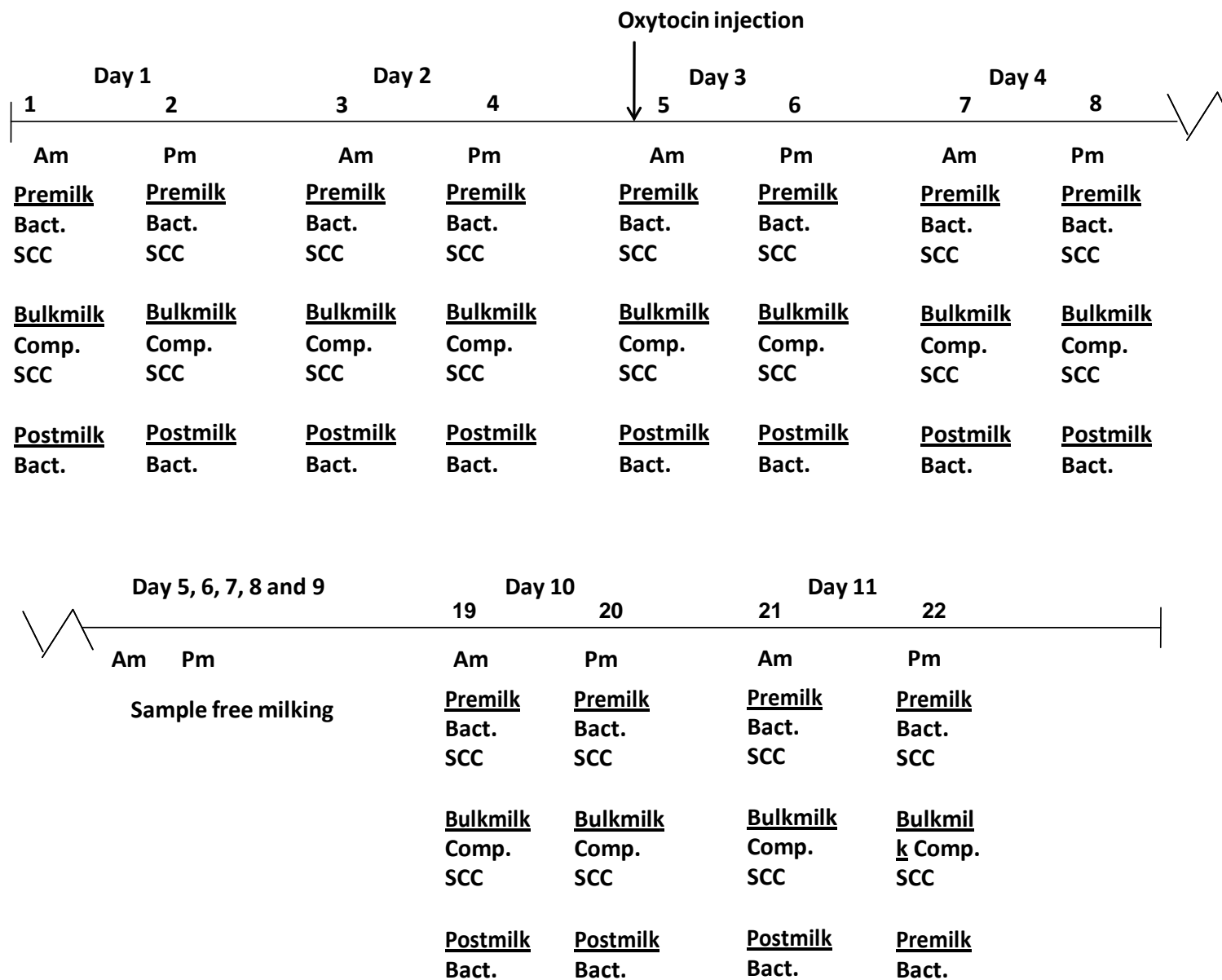
The trial started with four successive milking occasions, at am and pm for two days. During these milking occasions quarter milk samples for bacteriological examination of premilk and postmilk were taken aseptically (fig. 9). Quarter milk samples for analyses of SCC of fore milk were also taken. A composite milk sample taken from the True Test was also taken for analyses of the SCC and the milk composition. These samples were used as s.c basic values. For a schematic over view see fig. 10.



**Fig. 9.** Collection of aseptic milk samples (Jonsson, 2009).



At the next following milking (am day 3) 100 International units (IU) of oxytocin was injected intramuscularly. This high dose has been used previously as putative mastitis therapy (Guterbok *et al.* 1993, Knight *et al.* 2000). After two minutes a premilk sample was drawn, after that the cow was milked as normal and after the completed milking a post milk sample was drawn. A standardized procedure was used for all samplings. At the 3 next milkings (pm day 3, am and pm day 4) premilk and postmilk samples was drawn and a composite samples was taken as previous. Seven days after the oxytocin injection (day 10) premilk, postmilk and composite samples were taken at the am and pm milking, this was also done at the following day, day 11.



*Fig. 10. A schematic picture over the sampling days and the collection of the milk samples. The pre- and post milk samples were taken at udder quarter level.*

## Analyses of the samples

All milk samples (premilk and postmilk) for bacteriology was frozen (-18--20°C) directly after the sampling was done. The samples were then analysed later for *S. aureus* only, by using standard bacteriology, at National Veterinary Institute (SVA, Uppsala, Sweden). The True Test milk sample were analysed for SCC and milk composition (fat, protein, lactose and dry matter) in fresh milk. Quarter premilk samples was also analysed for SCC. The SCC of the milk were measured by using electronic fluorescence based cell counting technique (Fossomatic 5000,A/SN, Foss Electric Hilleröd Denmark) and the analysis of the composition was done with mid infra red spectroscopy (MilcoScan FT 120 Foss Electric, Hilleröd, Denmark).

## Statistical analyses

The samples that were taken before the oxytocin injection, were used as the cows own control value and the 4 cows that have a SCC less than 50 000 cells/ml were used as control animals for the whole treatment. The statistical analyses of the sample was made with PROC GLM in SAS 9.1 (SAS Institute inc., 2004) and also visualized by figures and tables made in Microsoft Office Excel 2007.

$$Y_{ijk} = \mu + \text{cow no}_i + \text{period}_j + \epsilon_{ijk}$$

$$Y = \text{SCC}$$

$$\text{Cow no} = \text{cow ID}, i = 1, 2, \dots, 14$$

$$\text{Period} = \text{milking occations}, j = 1-4, 5, 6, 7, 8 \text{ and } 19-22$$

## Results

In total 840 premilk samples were collected from each udder quarter and composite milk samples, taken from True Test, 5 samples from each milking. Of the aseptic samples 1344 were collected (8 each milking) although due to economic reasons only aseptic samples from the first sampling week from the HSCC group (640) were analyzed. From the LSCC group none of the aseptic samples were analyzed. One cow was included in the trial one milking later than the other, cow 1350.

## Bacteria

The results from the aseptic samples are compiled in table 3. Since the aseptic samples from the LSCC group were not analyzed these cows are excluded from the table. Two of the cows that had a negative aseptic sample before the oxytocin injection showed bacteria growth after the oxytocin injection. It was cow 1202 that had some type of *Streptococcus* and cow 1360 that had some sort of coliform bacteria. These two cows were the only cows that had bacteria in the aseptic samples that were not found before the oxytocin injection were given. The amount of positive bacteria samples from known bacterial infections did not increase after the oxytocin injection.

The distribution of the infections between the front and back quarters showed that the back quarters tend to be more infected. Seen over all cows there were 3 right front quarters infected, 3 left front, 4 right back and 5 left back quarters.

*Staphylococcus aureus* were found in one cow (1350) throughout the whole sample period, . Cow 1202 had one positive aseptic sample with *S. aureus* which was taken before the oxytocin injection was given. This bacteria was not detected from this udder quarter or any other udder quarter from this cow after the oxytocin injection was given.

Cow 1189 had positive aseptic samples for all four quarters before the oxytocin injection was given. After the injection it was only from the left back quarter the bacteria *S. spec* was found.

**Table 3.** Occurrence of bacteria in udder quarter milk before and after oxytocin treatment (100 IU). Samples taken before the oxytocin injection (milking 1-4) are called before Ox. and the samples taken after the oxytocin injection (milking 5-8) are called after Ox.

Cow Number	Udder quarter	Bacteria spices Before Ox.	After Ox.
1189	RF	<i>S. spec</i>	Neg.
	RB	<i>S. spec</i>	Neg.
	LB	<i>S. spec</i>	<i>S. spec</i>
	LF	<i>S. spec</i>	Neg.
1202	RF	Neg.	Neg.
	RB	Neg.	<i>Strept.</i>
	LB	Neg.	Neg.
	LF	<i>S. aureus</i>	Neg.
1288	RF	Neg.	Neg.
	RB	Neg.	Neg.
	LB	<i>S. spec</i>	<i>S. spec</i>
	LF	Neg./Cont.	Neg.
1344	RF	Neg./Cont.	Neg.
	RB	<i>S. spec</i>	<i>S. spec</i>
	LB	<i>S. spec</i>	<i>S. spec</i>
	LF	Neg.	Neg.
1350	RF	Neg.	Neg.
	RB	Neg.	Neg.
	LB	Neg.	Neg./Cont.
	LF	<i>S. aureus</i>	<i>S. aureus</i>
1360	RF	<i>Strept.</i>	<i>Strept.</i>
	RB	Neg.	Neg.
	LB	Neg./Cont	Neg./Cont
	LF	Neg./Cont.	Colif.
1368	RF	Neg.	Neg.
	RB	<i>S. spec</i>	<i>S. spec</i>
	LB	<i>Strept.</i>	<i>Strept.</i>
	LF	Neg.	Neg.
1376	RF	Neg.	Neg.
	RB	Neg.	Neg.
	LB	Neg.	Neg.
	LF	Neg.	Neg./Cont.
1377	RF	Neg.	Neg.
	RB	Neg.	Neg.
	LB	Neg.	Neg.
	LF	Neg.	Neg.
1385	RF	<i>S. spec</i>	<i>S. spec</i>
	RB	Neg.	Neg.
	LB	<i>S. spec</i>	<i>S. spec</i>
	LF	Neg.	Neg.

Note: Neg. = negative, Cont. = contaminated, *S. spec* = bacteria from the *Staphylococcus* genera, but not *S. aureus*, *Strept.* = bacteria from the *Streptococcus* genera, Colif. = a Coliform bacteria. RF= Right front, RB= Right back, LB= Left back and LF= Left front.

## Somatic Cell Count

The result of the mean SCC for the milk samples is shown in table 4. The result is divided in three groups, one for the HSCC group, one for the LSCC group and one for the two groups together. Seen over all cows, there was a significant difference between the first base value milkings (1-4) and the milking when the oxytocin was given (milking number 5). Although the biggest difference ( $p < 0.0001$ ) in SCC is for milking number 6, the milking after the oxytocin has been given.

**Table 4.** The mean SCC for the milk samples, the oxytocin injection was given at milking number 5

Mean SCC ( $10^3$ cells/ml)	Milking 1-4	Milking 5	Milking 6	Milking 7	Milking 8	Milking 19-22
HSCC group	244.42 <sup>a</sup>	504.77 <sup>b</sup>	1011.3 <sup>e</sup>	295.89 <sup>ac</sup>	375.03 <sup>bc</sup>	226.78 <sup>a</sup>
LSCC group	21.52 <sup>a</sup>	45.20 <sup>b</sup>	107.77 <sup>c</sup>	23.59 <sup>a</sup>	30.88 <sup>ab</sup>	28.39 <sup>ab</sup>
All cows	121.51 <sup>a</sup>	252.14 <sup>b</sup>	531.66 <sup>d</sup>	144.03 <sup>ac</sup>	183.10 <sup>bc</sup>	125.21 <sup>a</sup>

Note: Different superscript letters shows that there are a significant difference ( $p < 0.05$ ) between the probabilities.

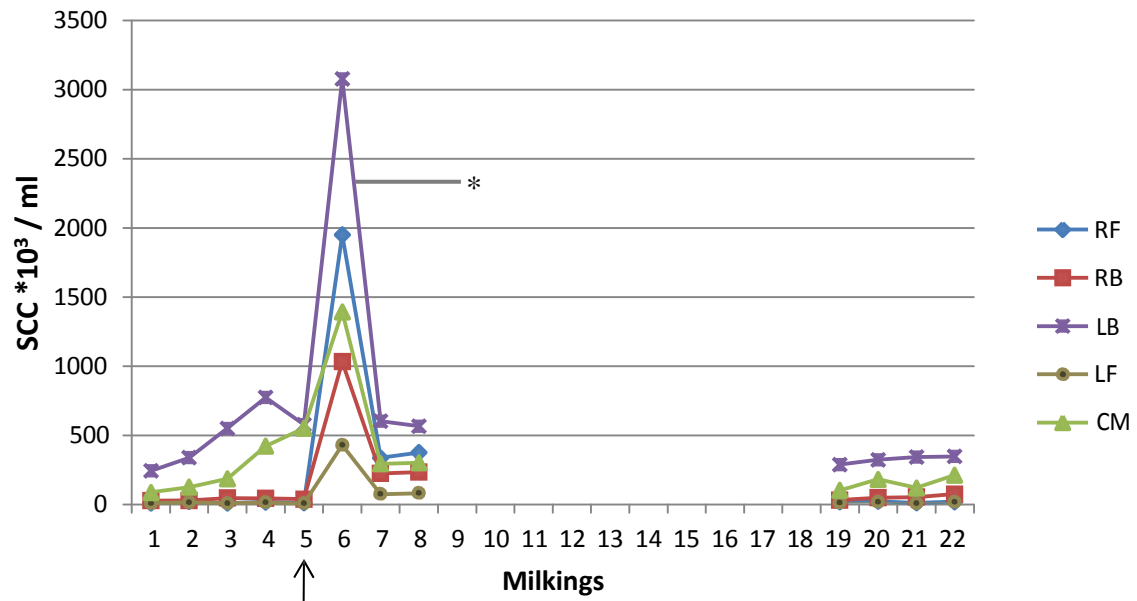
The cows individual SCC value reveals that the quarters that were infected with some kind of bacteria had in most case a higher base value of the SCC (milking 1-4) and the increased peak after the oxytocin injection is also higher than most quarters with a negative bacteria sample.

Cow 1198 (fig. 11) from the HSCC group shows the typical SCC pattern that the high oxytocin injection created, note that the scale in Y-range is not the same for all figures.

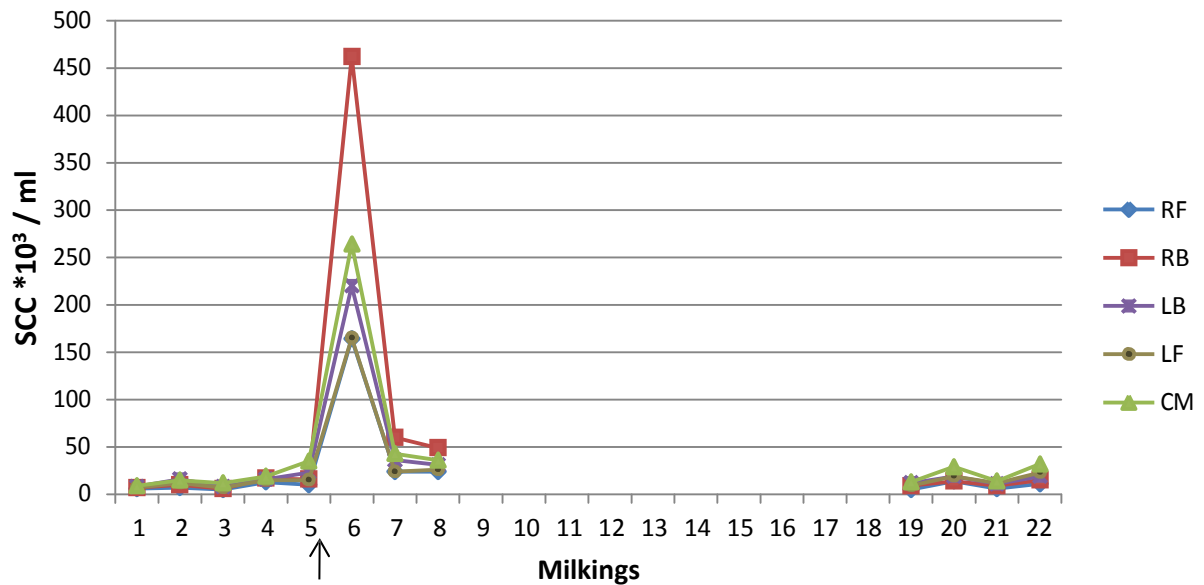
In the LSCC group the same SCC pattern exists although the SCC values is much lower, see fig. 12, where the result from cow 1382 is shown.

Cow 1344 is an example on how the level of the SCC is different depending on if the quarter has an infection or not, see fig. 13. Before the oxytocin injection the SCC for all quarters is relatively constant although the level for the infected quarters is much higher than the non infected are. At milking 6 the SCC for all quarters are increased and especially those for the two infected quarters. If the highest SCC peaks from each cow is compared with the result from the bacteria sampling (table 3), most peaks corresponds with a bacterial infection except cow 1376 (fig. 14) and cow 1377 (fig. 15). Cow 1376 has peak in the figure for the left back quarter but this is explained by the scale which is very low for all quarter. Cow 1377 has peak for the left back quarter which not was related to any found bacteria in the bacteria samples.

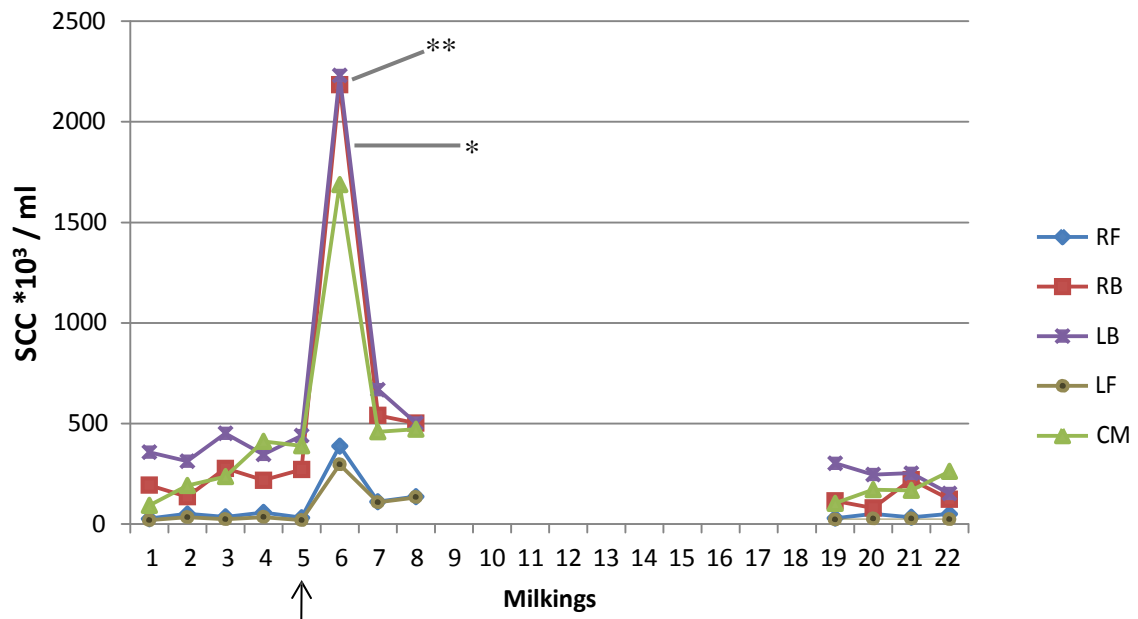
Cow 1350 which had *S. aureus* in the left front quarter did not had the highest SCC values at milking number 6, see fig. 16.



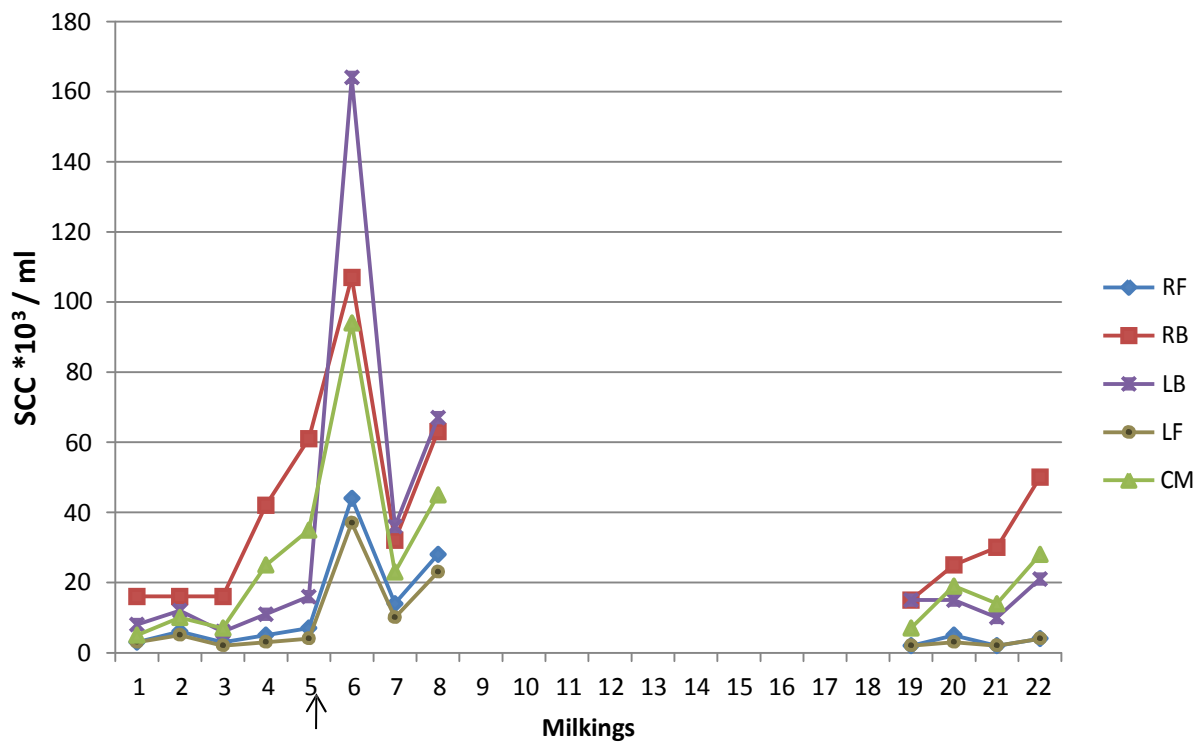
**Fig. 11.** The SCC from cow 1189 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \*= *S. spec.* was detected in this quarter before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



**Fig. 12.** The SCC from cow 1382 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. The value for right front has almost the same values as the values for left front and is by this difficult to visual. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

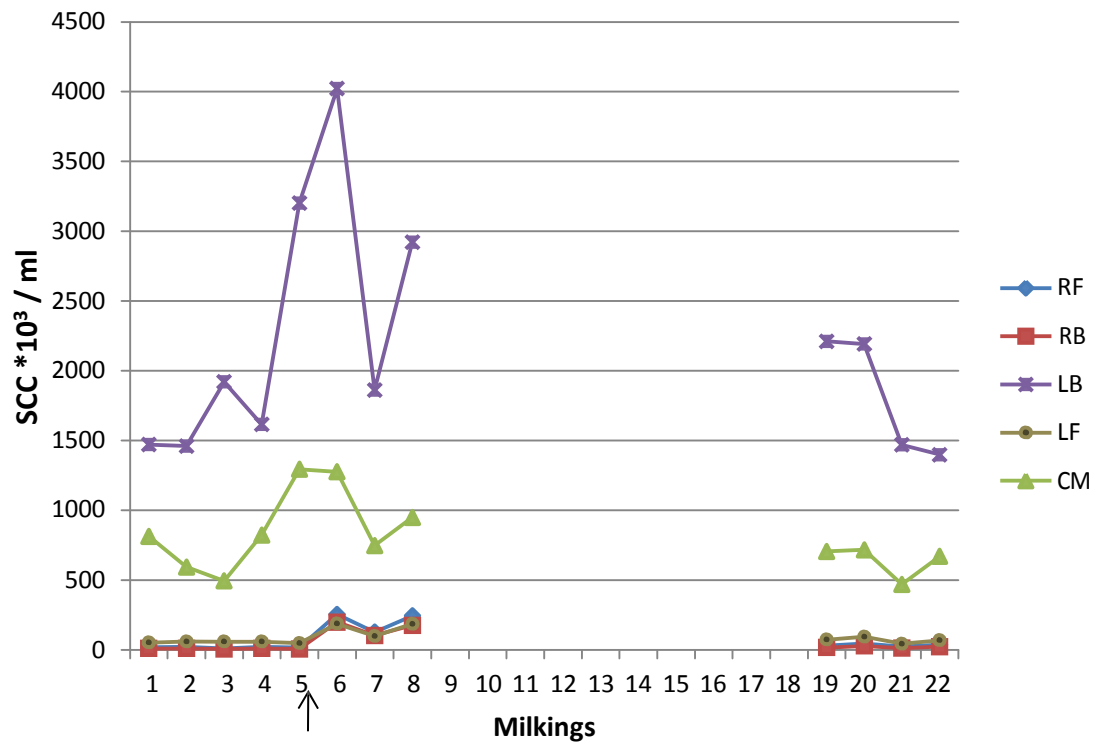


**Fig. 13.** The SCC from cow 1344 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \* and \*\*=*S. spec* was detected in these two quarters before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

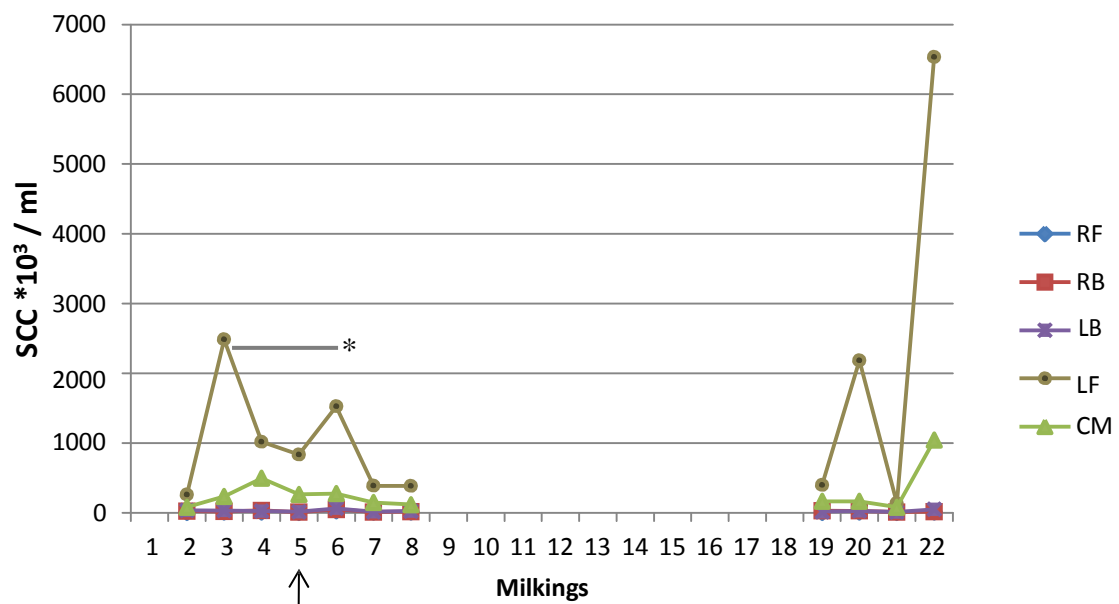


**Fig.14.** The SCC from cow 1376 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.





**Fig. 15.** The SCC from cow 1377 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. The value for right front has almost the same values as the values for right back and also as left front and is by this difficult to visual. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



**Fig. 16.** The SCC from cow 1350 during the trial period. This cow was included in the trial one milking later than the others. The value for right back has almost the same values as the values for left back and is by this difficult to visual. The arrow shows on milking 5 when the oxytocin injection was given. \*= *S. aureus* was detected from this quarter before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

## Milk composition

This following result is made on both the HSCC- and the LSCC-group as one group. This is because the result for each group separate gave the same result as the two of them together.

The composition of the milk was changed during the sampling period. Most of the changes appeared at the same milking as the oxytocin injection was given or at the next following milking, see table 5. At milking 5 the percent of fat was increased ( $p<0.0001$ ) compared to the other milkings. The percent of fat was decreased ( $p<0.01$ ) at the next morning milking, milking 7, compared to the other milkings. The percent of protein in the milk from milking 5 were lower ( $p<0.001$ ) compared with all the other milkings, except for milking 1-4 which only was significant different from milking five with  $p<0.05$ . Milking 6 had a higher ( $p<0.001$ ) percent of protein than all the other milkings. Milking 1-4 had lower ( $p<0.05$ ) percent of protein than milking 8. The percent of lactose at milking 6 is significant decreased ( $p<0.0001$ ) from all the other milkings. Milking 5 has a significant lower ( $p<0.01$ ) percent of lactose compared to milking 1-4.

**Table 5.** The mean value of the composition of the milk at the different milking opportunities, the oxytocin injection was given at milking number 5

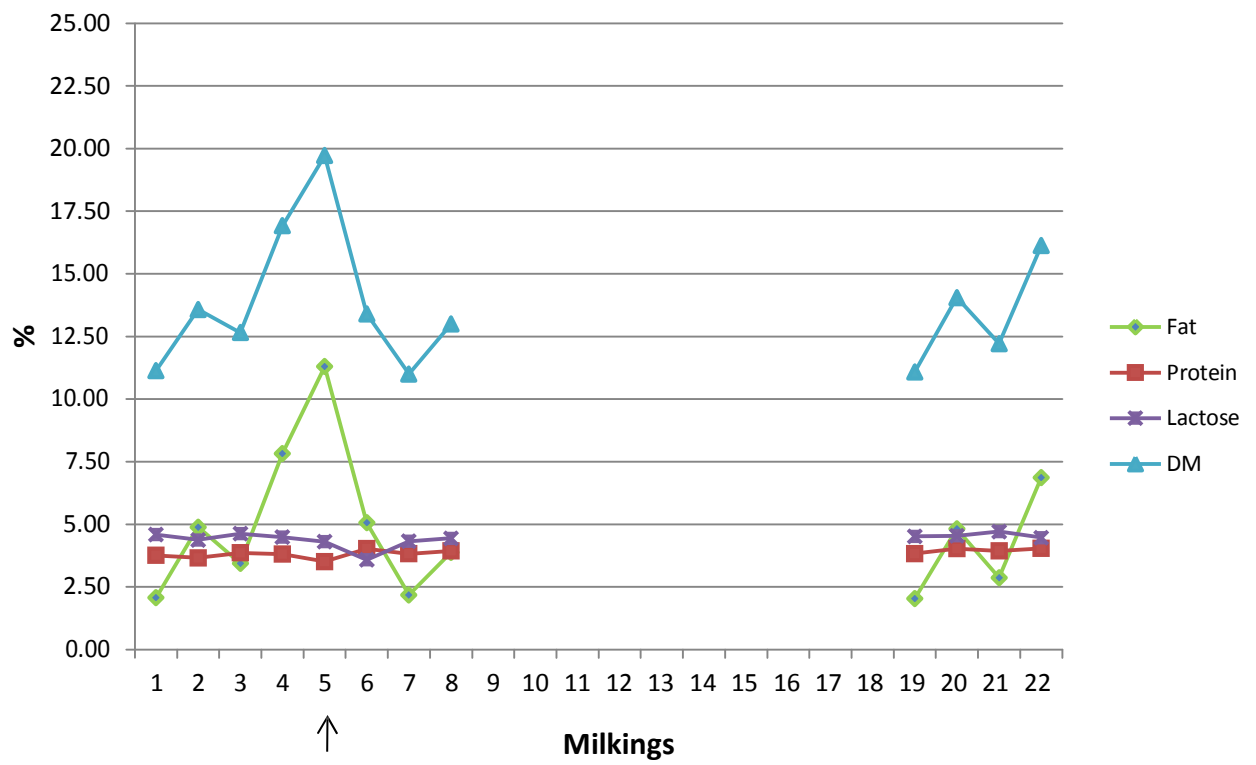
	Milking 1-4	Milking 5 AM	Milking 6 PM	Milking 7 AM	Milking 8 PM	Milking 19-22
Fat (%)	4.07 <sup>a</sup>	7.56 <sup>b</sup>	4.48 <sup>a</sup>	2.44 <sup>c</sup>	4.07 <sup>a</sup>	4.03 <sup>a</sup>
Protein (%)	3.77 <sup>b</sup>	3.67 <sup>a</sup>	4.04 <sup>c</sup>	3.84 <sup>bd</sup>	3.87 <sup>d</sup>	3.84 <sup>bd</sup>
Lactose (%)	4.66 <sup>b</sup>	4.54 <sup>c</sup>	4.32 <sup>a</sup>	4.61 <sup>bc</sup>	4.59 <sup>bc</sup>	4.65 <sup>bc</sup>

Note: Different superscript letters shows that there are a significant difference ( $p<0.05$ ) between the probabilities.

The changes in the milk composition for the individual cows is illustrated in fig. 17, by the milk composition from cow 1189. Figures for the other cows individual milk composition is attached as appendix 2. Figure 17 illustrate the average result from all samples very well, with the increased fat percent at milking 5 and the decreased percent of fat, protein and lactose at milking 6.

Figure 17 also illustrate a difference in the percent of fat between morning and afternoon milking, the afternoon milking is significant higher ( $p<0.01$ ) in fat for all the cows.

If the average of all the milkings (1-22) are compared to each other, milking 22 is higher ( $p<0.05$ ) in fat percent than the others. This is due to some cows that had a very high percent of fat at this milking, see figures in appendix 1 and appendix 2.



**Fig. 17.** The composition of the milk from cow 1189. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.

## Discussion

### Bacteria and SCC

The oxytocin injection gave a significant increased SCC-value as expected (Allen 1990; Sandholm a 1995). In general the SCC from each udder quarter follows a base line with a peak at the milking after the oxytocin injection. Some of the udder have a base line above 250 000 cells/ml and also looking at the separate udders quarters most of them has a base line under 50 000 cells/ml. These values are often used as threshold value for differing between a healthy or an infected udder (Schukken *et al.* 2003). Most of the increased SCC values can be explained by the result from the antiseptic samples, where bacteria was detected in the same udder quarter. It was only two quarters (from different cows) that did not seem to have any bacteria according to the aseptic samples that were taken. These quarters can still have bacteria, but the bacteria was not detected in the milk sample. Cow 1202 is an example, of the difficulties to detect occurrence of bacteria in milk. This cow did only have one aseptic sample that was positive for *S. aureus* during the sampling time, see Appendix 1, fig. 1. The samples that were taken later on, also gave a negative result.

Cow 1350 (fig.16) , which had *S. aureus* throughout the entire sampling period shows that the quarter that has a *S. aureus* infection can vary a lot in the SCC. It also showed that a *S. aureus* infected udder quarter does not have to have a SCC value that is very high. At some milkings the SCC were around 250 000 cells/ml. The *S. aureus* infected udder quarter does not seem to respond to the oxytocin injection. The milking after the injection, where most cows had a clear peak in the SCC values, this udder quarter only had a small peak and it even had higher peaks at several other milkings. The SCC value from the cow's other quarters shows that the physical respond to the oxytocin injection. Although the *S. aureus* infected udder quarter did not had a distinct peak, this might be due to the bacterial infection. The influence that the *S. aureus* has on that udder quarter and the immune defense system is bigger and creates a higher SCC than the oxytocin injection does. By this the oxytocin peak is there but it is not so well pronounced since the *S. aureus* has already triggered the immune system and a high SCC value. The two cows 1202 and 1368 shows a similar pattern for their udder quarters that was infected with *Strept.* There is a peak at the 6<sup>th</sup> milking but there is also peaks at other milkings that the bacteria is causing, see figures 1 and 4 in Appendix 1. The reason to why not all infected udder quarters, from all of the participated cows, had SCC peaks other than after the oxytocin injection, might be that infections are more of the chronically type than those at cow 1350, 1202 and 1386.

Even though the result of the antiseptic samples did not show any udder quarter with *S. aureus* that was not detected before the oxytocin injection, it is still possible that the oxytocin injection started a shedding of *S. aureus* in some quarters according to the results presented by Daley *et al.* 1991. The method that were used in the present trial, to take samples before and after milking and for several days in a row is proven to give a high chance of finding the bacteria (Sears *et al.* 1991). Also freezing the samples before they are being analysed is found to increase the number of detected bacteria. The reason to this is that the freezing damage the macrophage and neutrophils in the milk and the phagocytized bacteria can be detected (Godden *et al.* 2002). Despite these methods were used no *S. aureus* were detected, which can be due to several reasons like wrong time of taking the antiseptic samples, a higher shedding rate might occurred at another time of the milking phase, the amount of oxytocin that were injected could be wrong or the time between the oxytocin injection and the milking.

A quarter can be infected with more than one type of bacteria at the same time (Young *et al.* 2001). It is also shown that bacteria have a shedding pattern (Daley *et al.* 1991; Struder *et al.* 2008). But how does the different bacteria strains interact with each other? Does the shedding pattern become synchronized because the two strains are attached by the same immune defense system? Is it possible that the one strain become more dominant? If that is the case, it is possible that *S. aureus* can be hidden by other bacteria. It has also been shown that *S. aureus* does not have to increase the SCC when it has entered in to the chronically phase (Studer *et al.* 2008). Since the amount of shedded *S. aureus* bacteria can be very low and the SCC does not have to be high it is possible that some of the udder quarters that had a high SCC and also had a detected bacteria which was not *S. aureus*, might have had an earlier infection by *S. aureus*. But the new stain hides the old *S. aureus* infection. This also shows on that in order to be able to detect *S. aureus* a more sensitive tool than measuring the SCC and spreading aseptic milk samples on an agar plate is necessary.

A problem with the method that the aseptic milks samples is taken with, is that a large amount of milk increase the chance of catching the bacteria but it also dilutes the few bacteria that is in the milk. A method that gives a high number of bacteria per milliliters of milk makes the detection easier (Godden *et al.* 2002). But reducing the amount of milk and still keeping the bacteria in the sample, alive and not contaminated is not possible today, with the aseptic method. Although the analyze method where the bacteria's DNA is measured instead might make it possible with this kind of bacteria concentration, since that method is not sensitive to contamination.

The aseptically taken milk samples are in the laboratory enriched on a blood agar plate. This is made to start the assumed bacteria to grow. The *S. aureus* which is assumed to be shaped as microabscesses is a non dividing stage for the bacteria. There might be so that the *S. aureus* is in the milk but it is not able to grow on the blood agar plate and by this creates a negative analyze result.

It is impossible to say if the quarters with mixed bacteria growth is due to contamination, or if there is a bacteria from the inside of the quarter as well, but it is being "hidden". Sears *et al.* 1991 found that contaminated aseptic bacteria samples were more common in samples that were taken before the milking was done. The result from this study does not support that theory. Cow 1189 has a lot of contaminated aseptic samples. The reason to that might be that she was one of the first cows I took aseptic samples from and she was this type of cow that always gets dirty, she had also problems with standing still. There were more back quarters infected than front quarters. A reason to this can be that they are more often dirty due to that they are closer to where the feces lands.

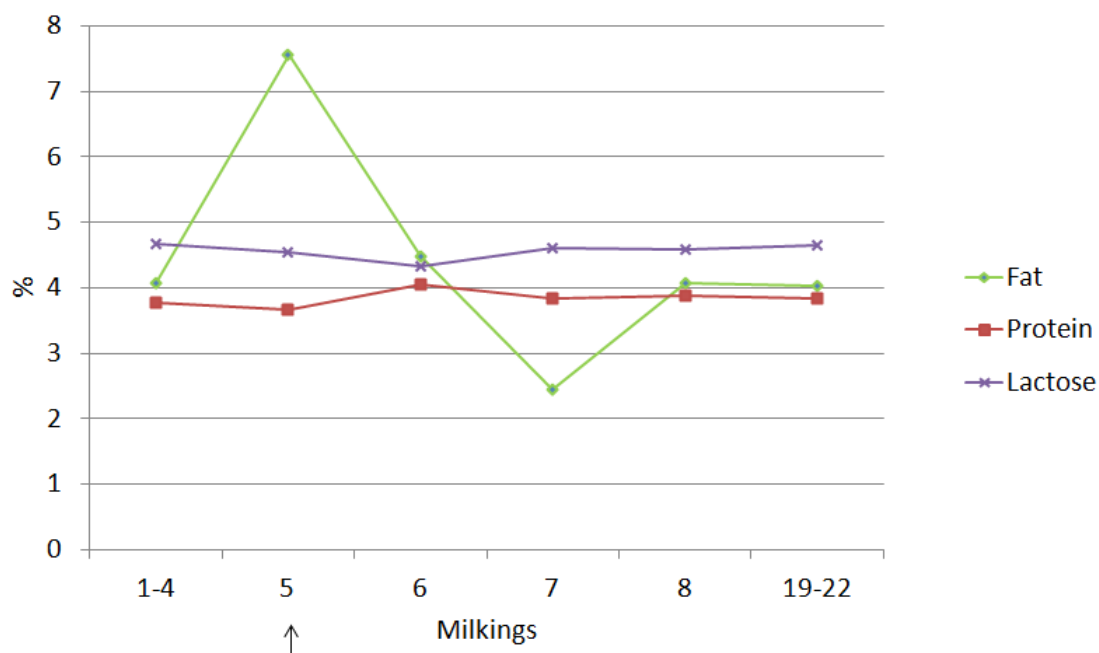
The increased SCC at the milking after the oxytocin injection indicates an inflow through the tight junctions. This also shows on a temporary damage on the alveoli and the tight junctions which was what it was supposed to create. But it is also important to keep in mind that the amount of milk at this milking was less than normal and this will also make the concentration of SCC higher.

If this trial would be repeated the way of selecting the cows could be done a bit differently. The HSCC group would only consist of cows that has a SCC above 250 000 cells per ml but without any other known bacteria or with some of the pre aseptically milk samples positive for *S. aureus*. This is to make it possible to only see the oxytocin effect on the *S. aureus*, not on udder pathogens in general. A higher amount of participating cows would also create a

more secure evaluation of the method. But all of these factors need a bigger budget and more time than what is possible for thesis on master level.

## Milk composition

The milking when the oxytocin injections were given (milking number 5) and the residual milk was collected was expected to have a higher amount of fat than normal (Allen 1990; Gorewit & Sagi 1984). In table. 5 it is shown that so was also the case. The amount of fat is unusual low at milking number 7 and that can be seen as a prolonged effect of milking number 5. If the result of the milk composition of the cows is plotted in a figure this becomes very clear. see fig. 18. The udder does not have enough time to produce fat both for excretion milk and also to the residual milk. It might seem strange that this is not shown on the 6<sup>th</sup> milking, but the total amount of milk is much lower at this milking so a reduction in total amount of fat does not affect the concentration of fat.



**Fig. 18.** *The composition of the milk from all cows.*

The concentration of protein does not follow the changes of the fat concentrations. At the second milking after the oxytocin injection (milking number 6) the concentration of protein has increased, a reason for that could be that the high dose of oxytocin has opened up the tight junctions and by this open up the passage between blood and milk. More somatic cells and sodium than normal is then entering the milk from the blood. The increased amount of somatic cells is shown as a higher protein concentration. The increased amount of protein is also occurring at the same milking as the big increasing of SSC which also gives support to this theory. A decrease in protein concentration was expected at the 6<sup>th</sup> milking (Allen 1990; Gorewit & Sagi 1984).

The low amount of lactose after the oxytocin injection indicates that the tight junctions was more permeable than usually due to the oxytocin effect. The ions can pass through the blood milk barrier and the osmosis makes the lactose go from the milk to the blood (Allen 1990).

## **Conclusion**

The hypothesis with this study was that the *S. aureus* was hidden in microabscesses in the udder tissue and that the high dose of oxytocin would create physical changes that would shed out the *S. aureus*. The result from this study does not bring any evidence that can support that theory.

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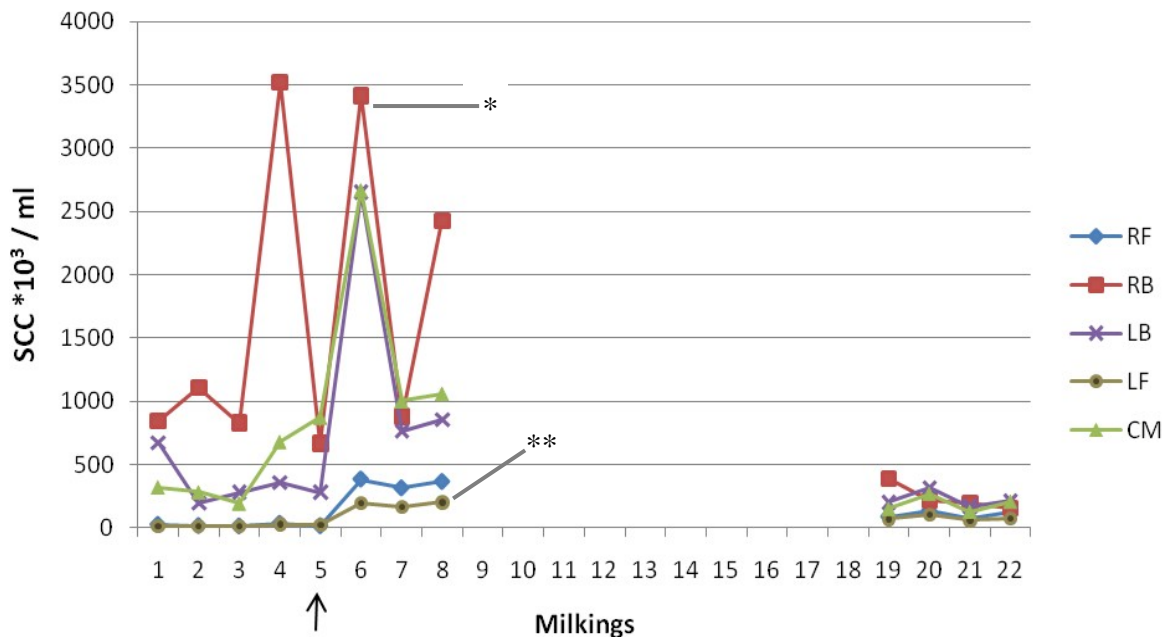
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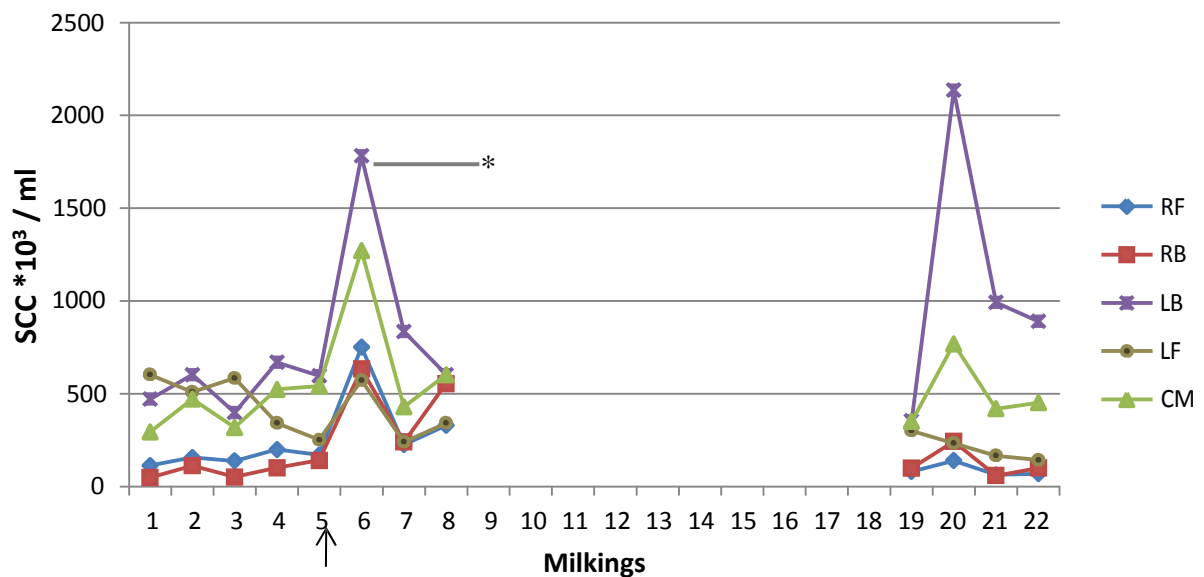
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## Appendix 1

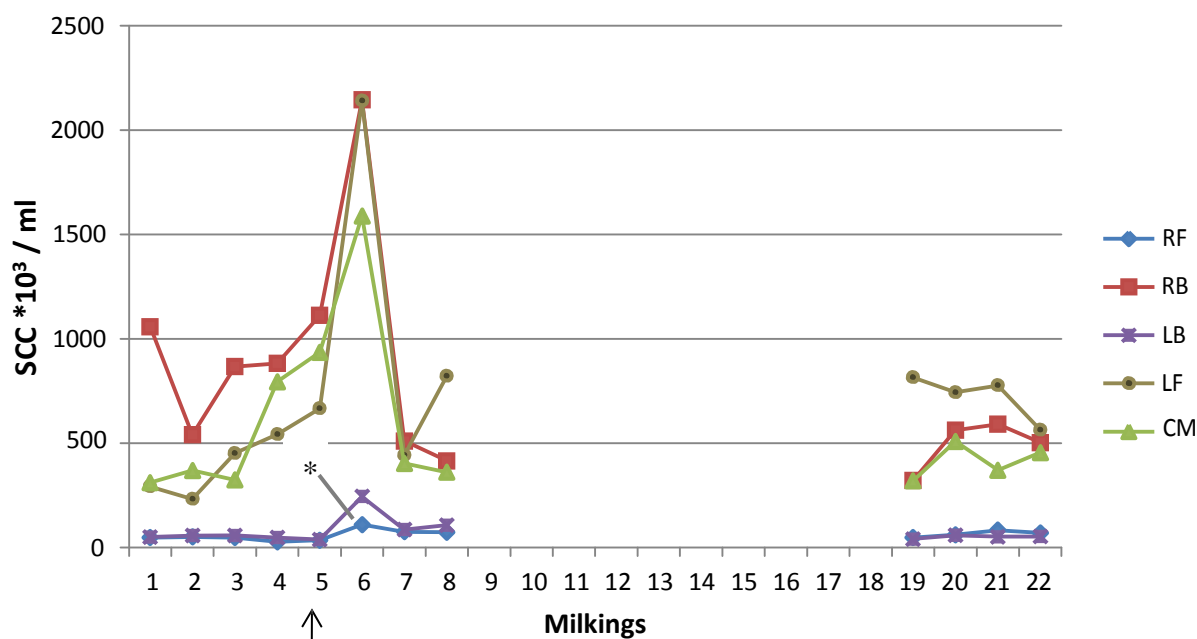
The individual SCC for the HSCC group which has not been presented above, is shown in figure 1-5, note that the scale in Y-range is not the same for all figures.



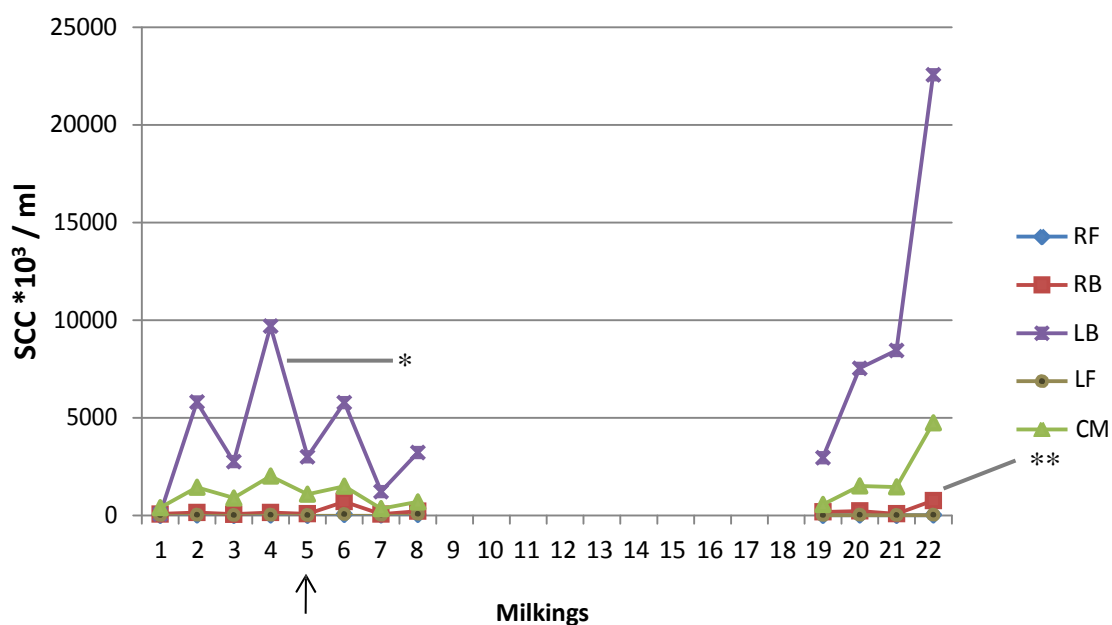
**Fig. 1.** The SCC from cow 1202 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \*= Strept. was detected in this quarter before and after oxytocin was given. \*\*= S. aureus was detected in this quarter before oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



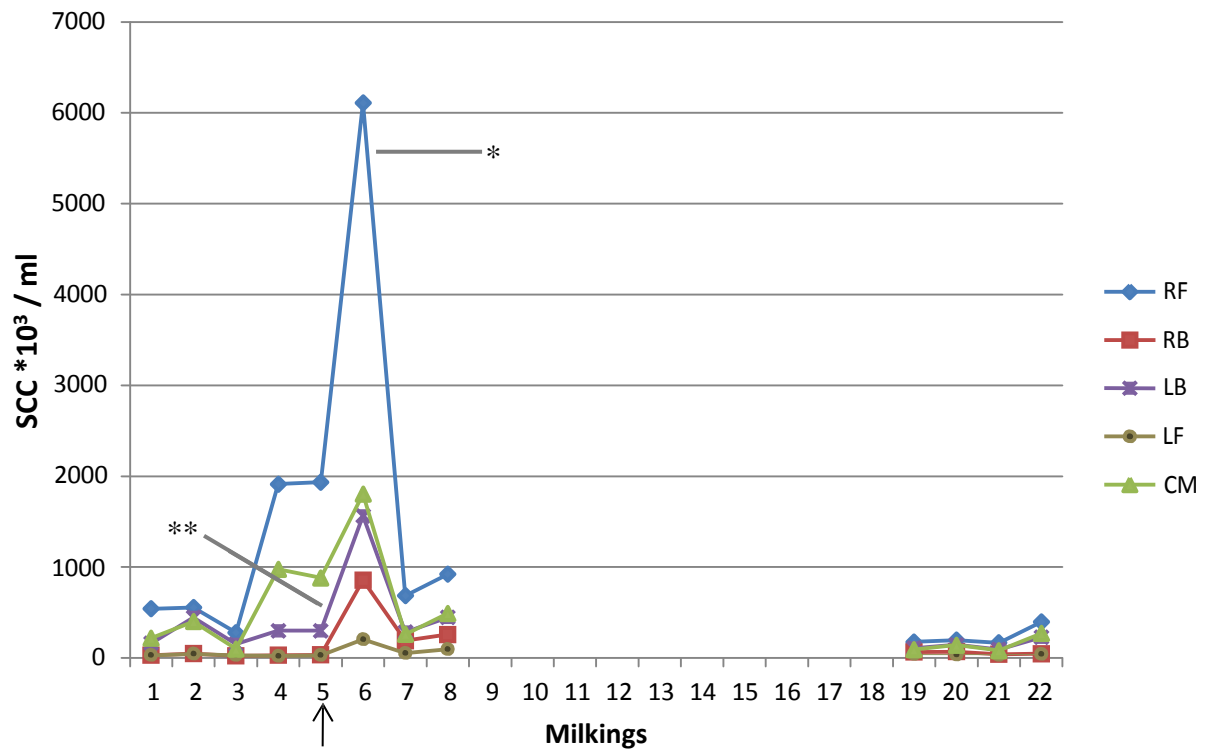
**Fig. 2.** The SCC from cow 1288 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \*=S. spec was detected in this quarter before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



**Fig. 3.** The SCC from cow 1360 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \*=Strept. was detected in this quarter before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

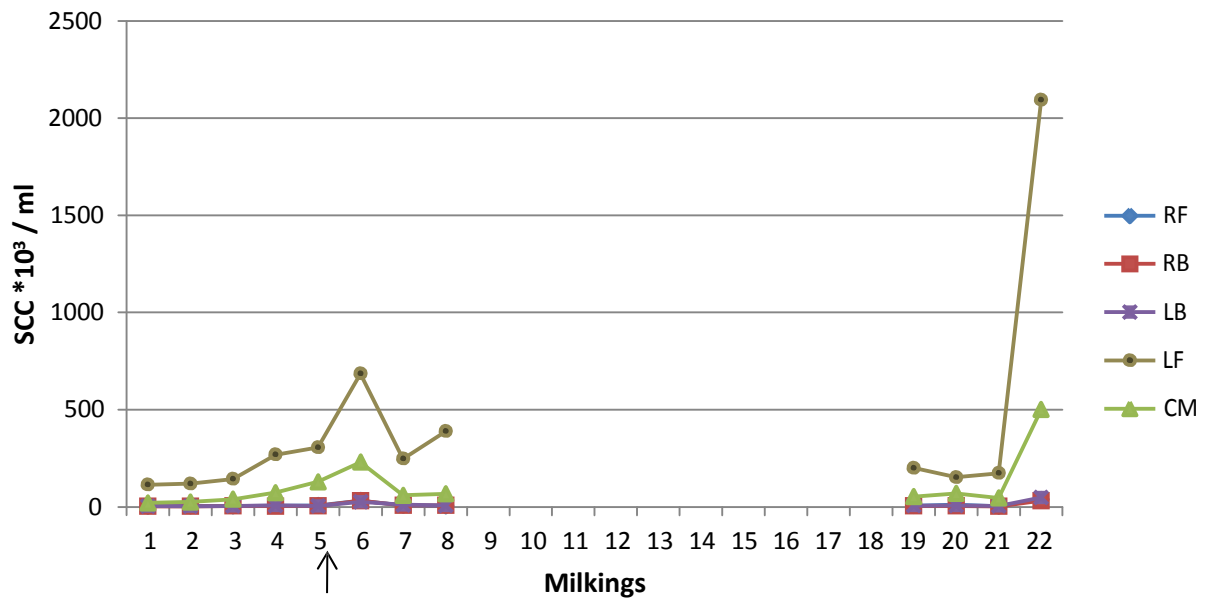


**Fig. 4.** The SCC from cow 1368 during the trial period. The value for right back has almost the same values as the values for left front and is by this difficult to visual. The arrow shows on milking 5 when the oxytocin injection was given. \*= Strept. was detected in this quarter before and after oxytocin was given. \*\*= S. spec was detected in this quarter before and after oxytocin was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

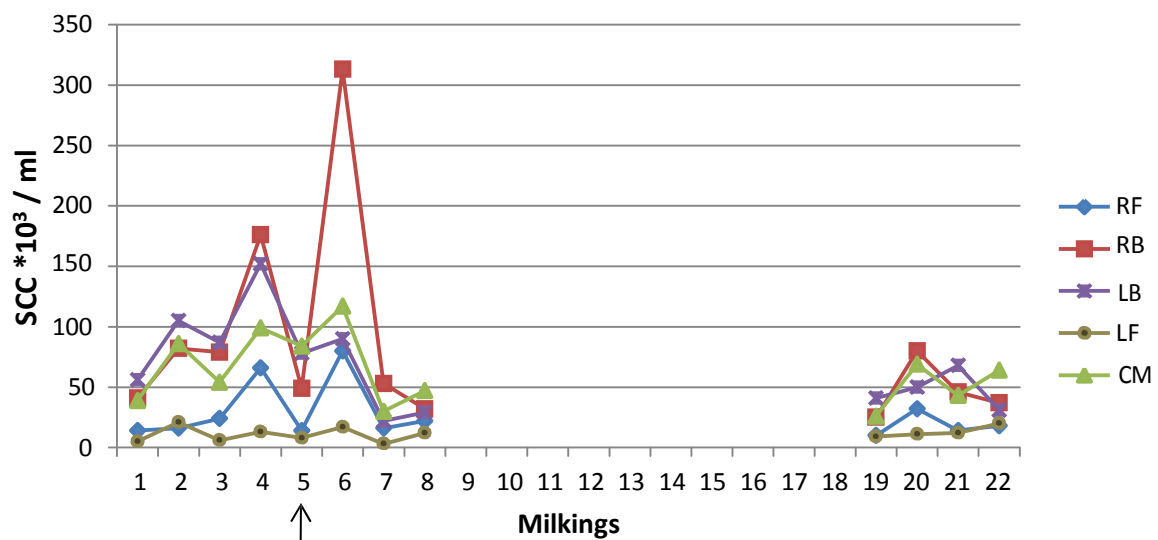


**Fig. 5.** The SCC from cow 1385 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. \* and \*\* = *S. spec* was detected in these two quarters. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

The SCC for the LSCC group which has not been presented above, is shown in figure 6-8. Since the result from these cows belongs to the LSCC group there is no result from any bacterial samples, note that the scale in Y-range is not the same for all figures.

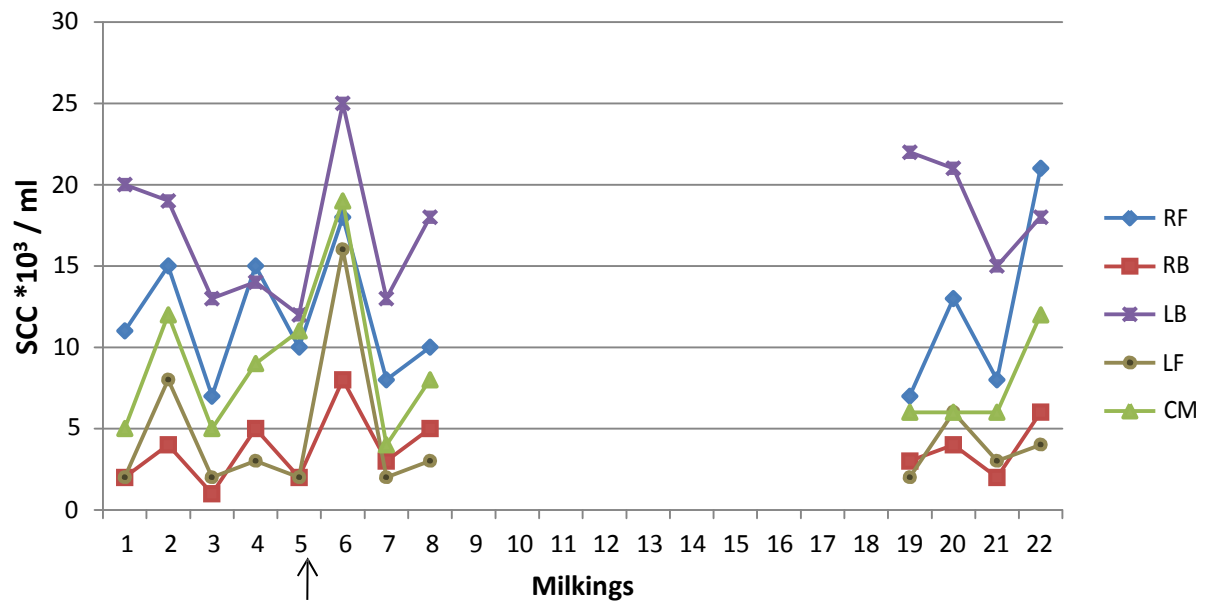


**Fig. 6.** The SCC from cow 1262 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. The value for right front has almost the same values as the values for right back and also as left back and is by this difficult to visual. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



**Fig. 7.** The SCC from cow 1290 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.



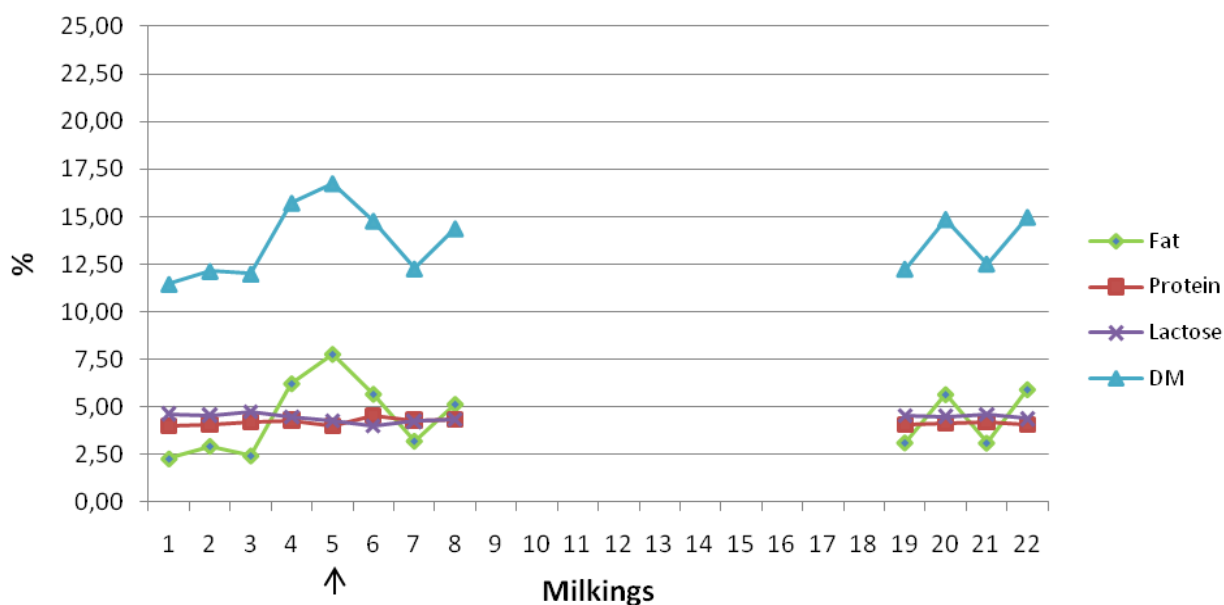


**Fig.8.** The SCC from cow 1296 during the trial period. The arrow shows on milking 5 when the oxytocin injection was given. RF= Right back, RB= Right front, LB= Left back, LF= Left front and CM= Composite milk.

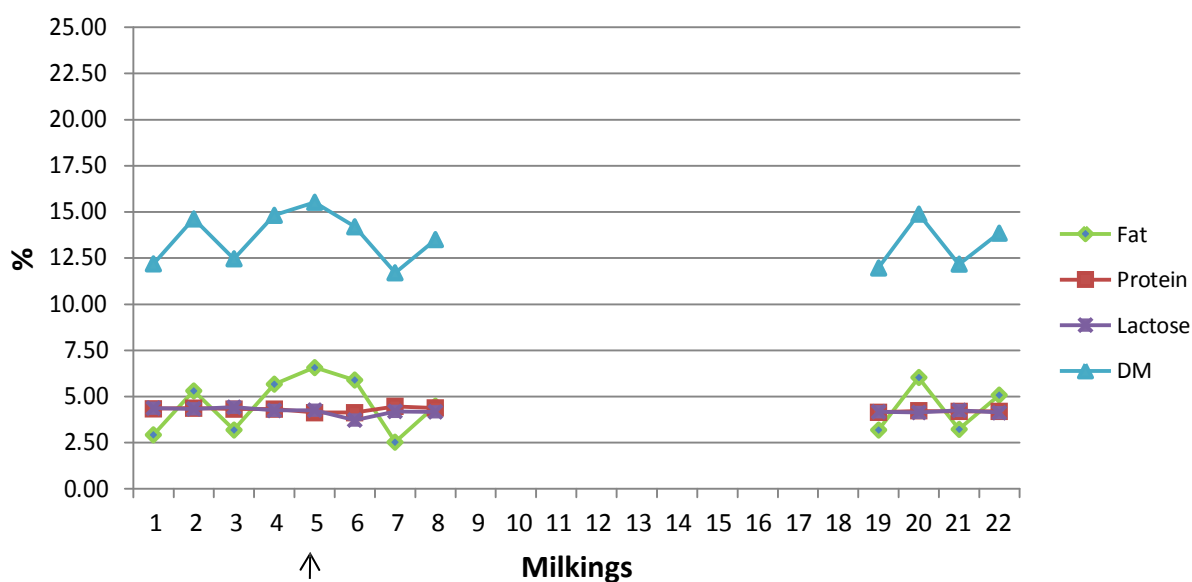
## Appendix 2

### Milk composition

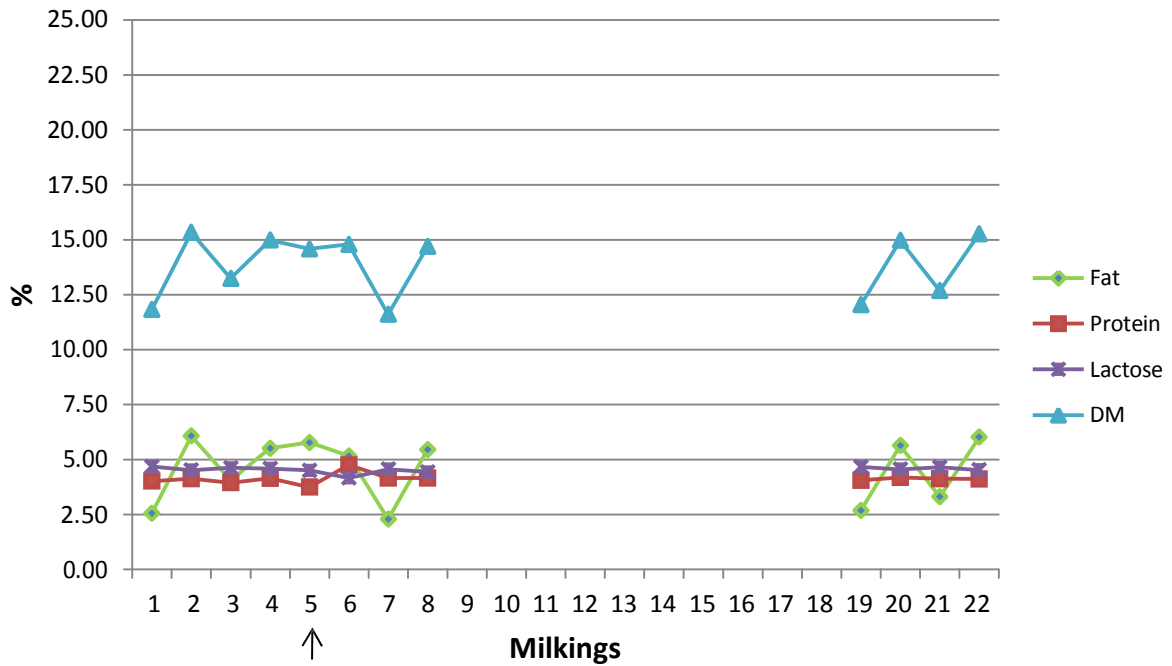
The milk composition of the HSCC group is shown in figure 1-9.



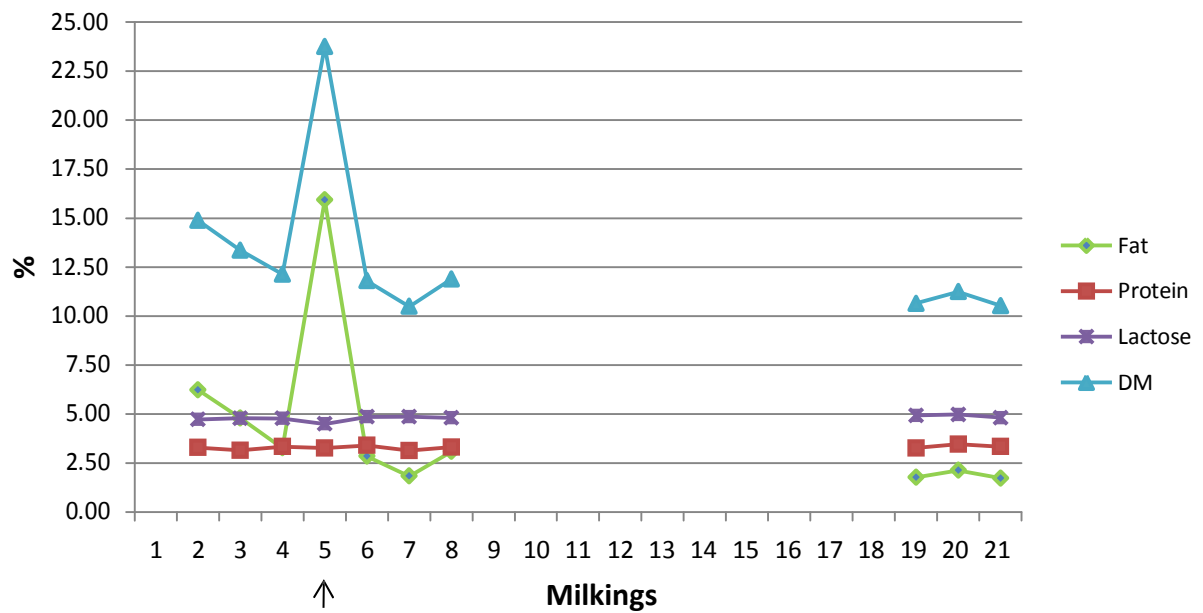
**Fig. 1.** The composition of the milk from cow 1202. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



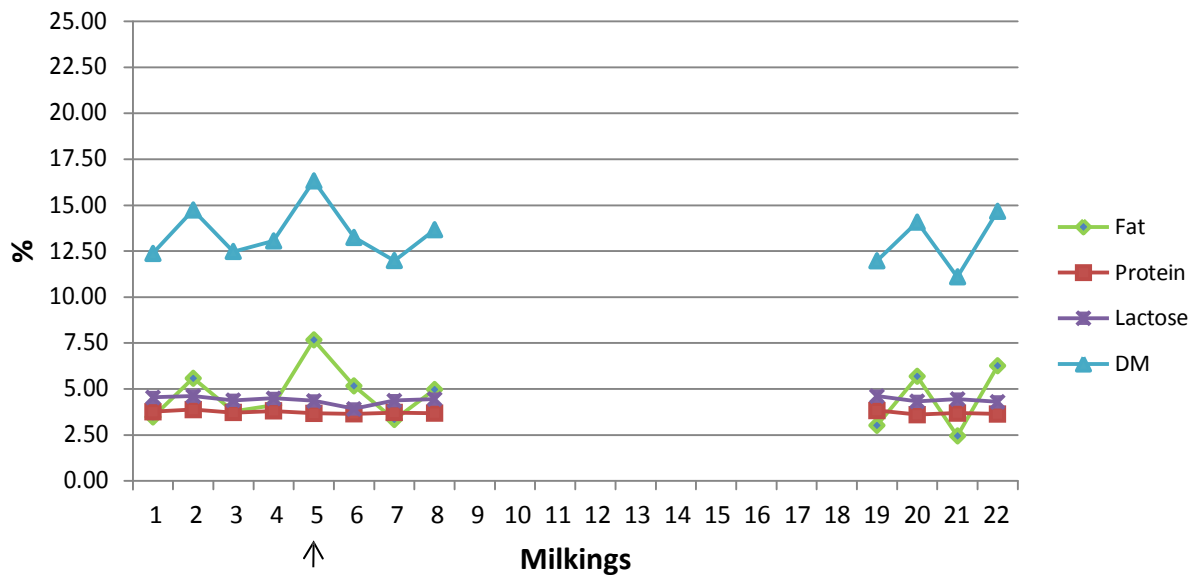
**Fig. 2.** The composition of the milk from cow 1288. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



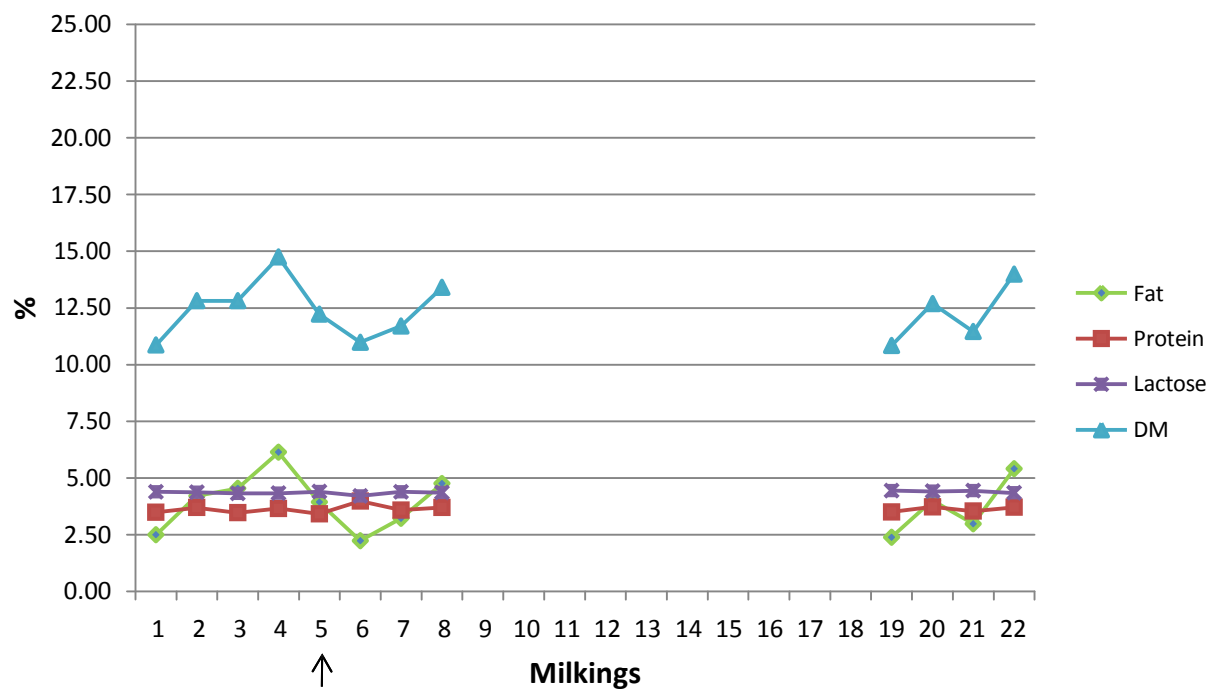
**Fig. 3.** The composition of the milk from cow 1344. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



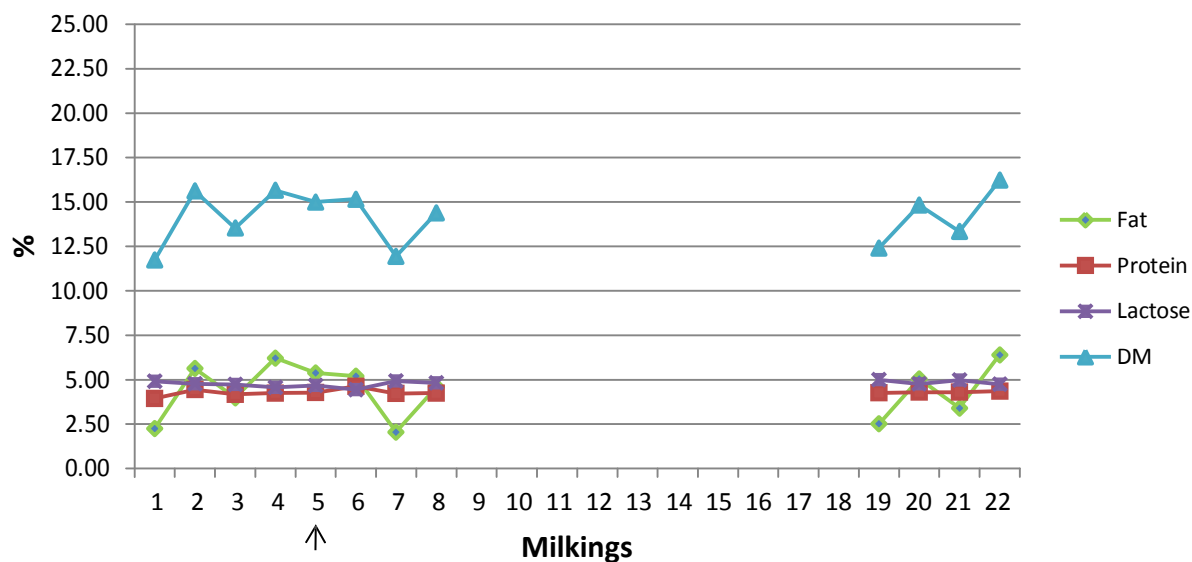
**Fig. 4.** The composition of the milk from cow 1350. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



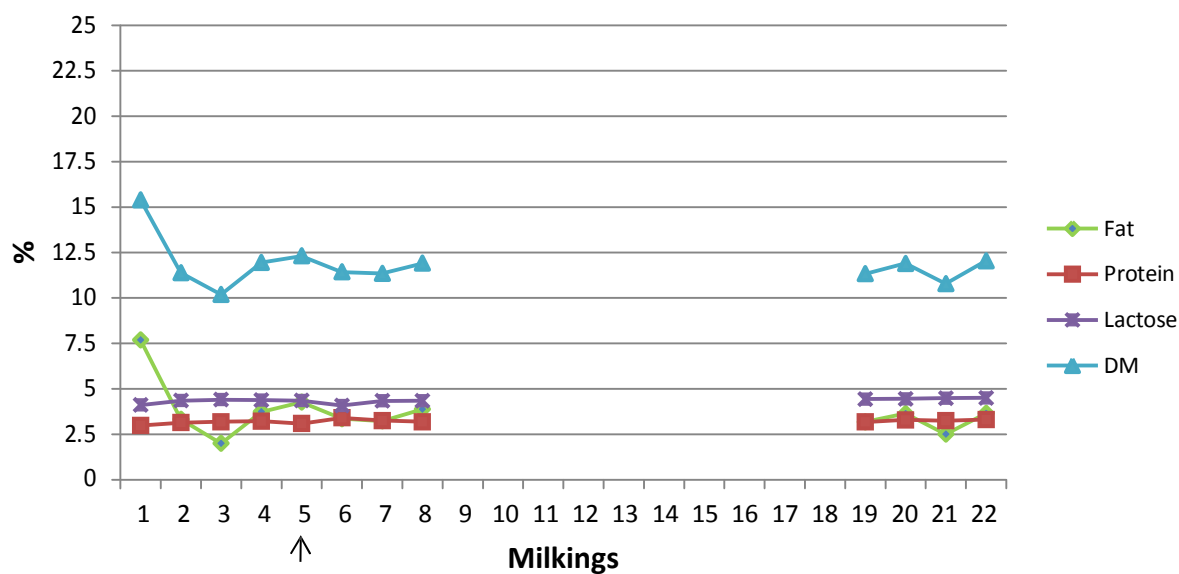
**Fig. 5.** The composition of the milk from cow 1360. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



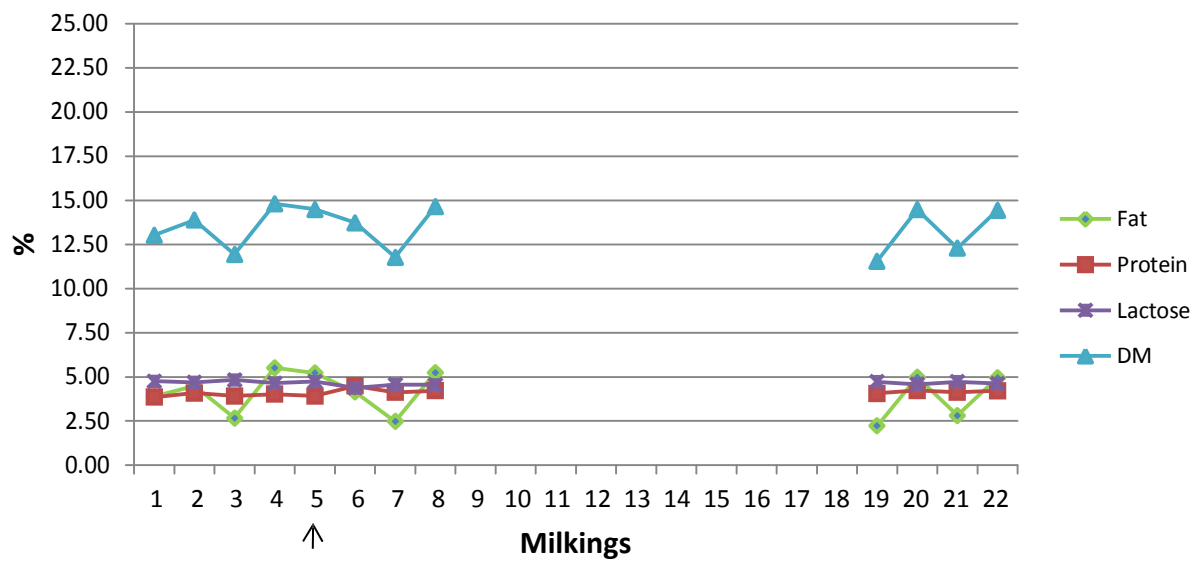
**Fig. 6.** The composition of the milk from cow 1368. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



**Fig. 7.** The composition of the milk from cow 1376. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.

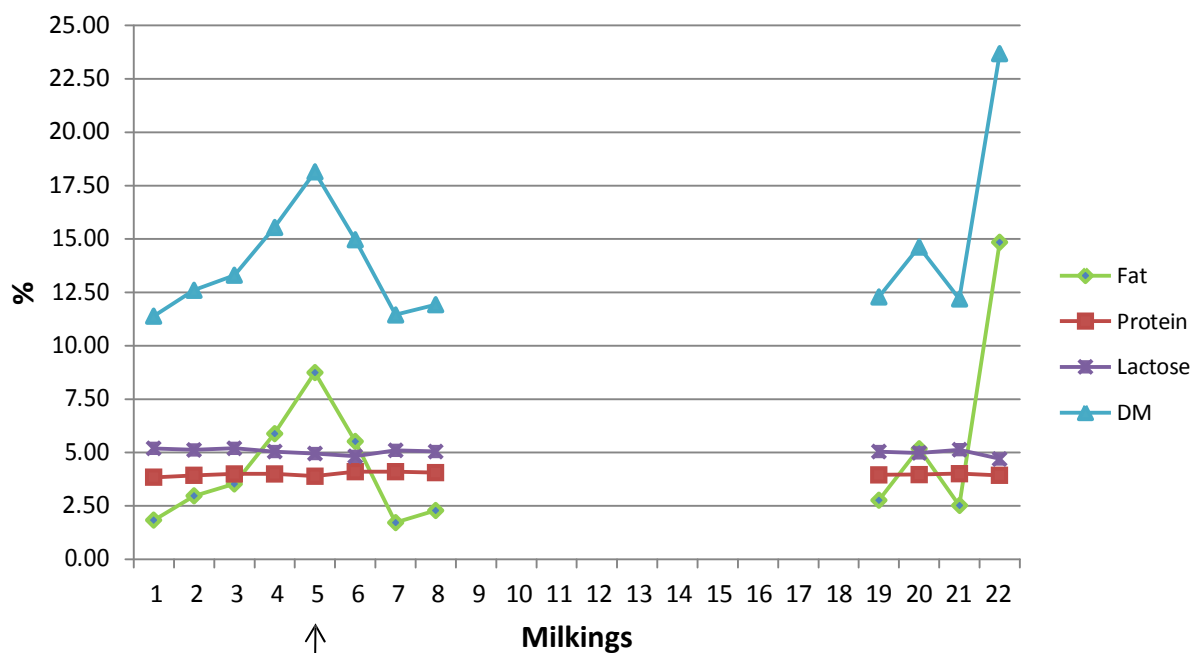


**Fig. 8.** The composition of the milk from cow 1377. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.

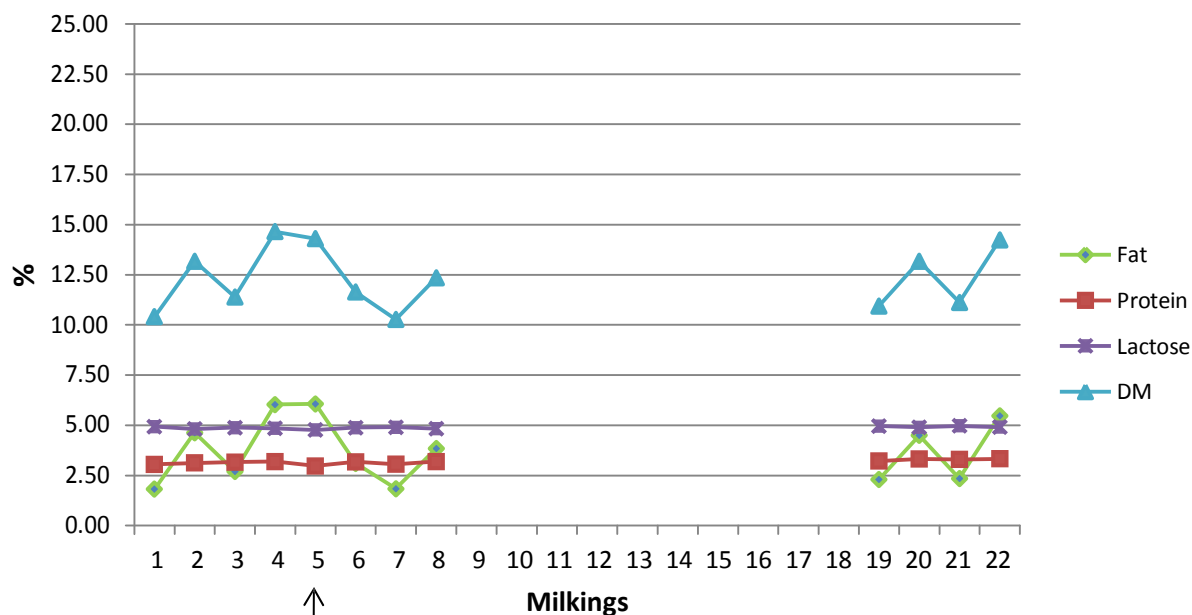


**Fig. 9.** The composition of the milk from cow 1385. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.

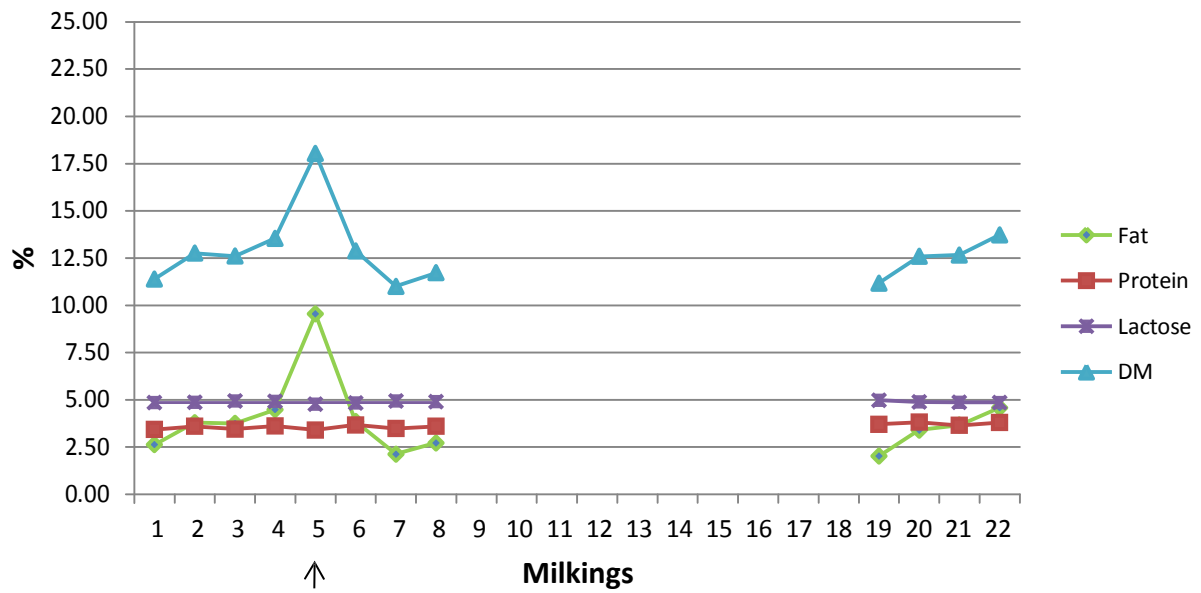
The milk composition for the LSCC group is shown in figure 10-13



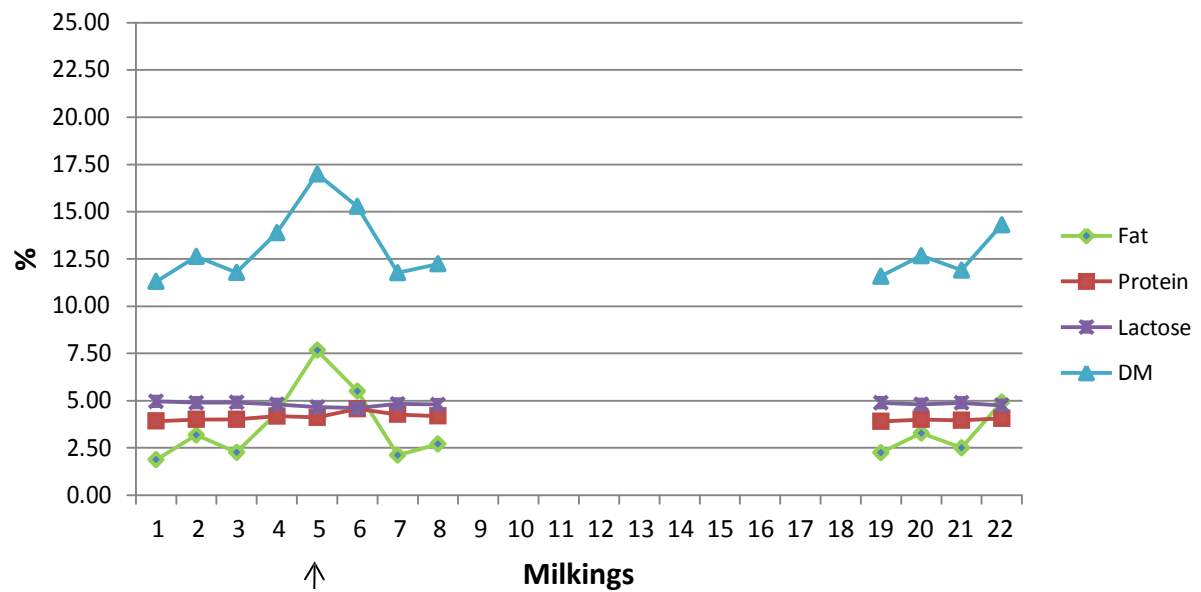
**Fig. 10.** The composition of the milk from cow 1262. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



**Fig. 11.** The composition of the milk from cow 1290. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



**Fig. 12.** The composition of the milk from cow 1296. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



**Fig. 13.** The composition of the milk from cow 1382. The arrow shows on milking 5 when the oxytocin injection was given. DM stands for dry matter.



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